National Toxicology Program
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Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR EXPERT PANEL REPORT on the REPRODUCTIVE and DEVELOPMENTAL TOXICITY of ETHYLENE GLYCOL

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PREFACE

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A REPORT OF THE CERHR GLYCOLS EXPERT PANEL

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Note to Reader:

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site (http://cerhr.niehs.nih.gov/). The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations in the synopses of the study. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by the Panel.

Abbreviations

ACC	American Chemistry Council
ACGIH	American Conference of Governmental Industrial Hygienists
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
ATSDR	Agency for Toxic Substances and Disease Registry
ANOVA	analysis of variance
AUC	area under the concentration versus time curve
bw	bodyweight
C	Celsius
$^{13}C_2$	carbon-13
^{14}C	carbon-14
CAS RN	Chemical Abstract Service Registry Number
Cmax	Maximum concentration
CERHR	Center for the Evaluation of Risks to Human Reproduction
Cl_{oral}	clearance after oral dosing
Cl_{total}	total clearance
cm	centimeter
CNS	central nervous system
$C0_2$	carbon dioxide
EG	ethylene glycol
F	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FSH	follicle stimulating hormone
g	gram
Ga_2O_3	gallium oxide
GA	glycolic acid
GC	gas chromatography
gd	gestation day
GLP	Good Laboratory Practices
H_2O_2	hydrogen peroxide
HazDat	Hazardous Substance Release and Health Effects Database
Hg	Mercury
hr	hour
HPLC	high pressure liquid chromatography
HSDB	Hazardous Substances Data Bank
IV	intravenous
kg	kilogram
K_m	Michaelis constant
K_{ow}	octanol-water partition coefficient
L	liter
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LOAEL	lowest observed adverse effect level
M	male
m^3	meters cubed
mg	milligram
mL	milliliter
MLD	minimum lethal dose

mm	millimeters
mmol	millimole
mol	mole
MRT_{∞}	mean residence time
MS	mass spectrometry
mw	molecular weight
NA	not analyzed
$NaHCO_3$	sodium bicarbonate
no.	number
ng	nanogram
ND	not determined
NIEHS	National Institute of Environmental Health Sciences
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NOES	National Occupational Exposure Survey
NIOSH	National Institute of Occupational Safety and Health
NS	not specified
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PET	polyethylene terephthalate
pnd	postnatal day
ppm	parts per million
RCF	regenerated cellulose film
sc	subcutaneous
SCE	sister chromatid exchange
SD	standard deviation
$t_{1/2}^{\beta}$	half life of elimination
TFT	trifluorothymidine
Tmax	time to maximum blood levels
TRI	Toxics Release Inventory
TWA	time weighted average
$U_{\infty}^{\text{ethylene glycol}}$	percent dose excreted as ethylene glycol in urine
USEPA	United States Environmental Protection Agency
V_{max}	maximal velocity of metabolism
w/v	weight per volume
µg	microgram
µL	microliter
µM	micromolar
µmole	micromole

TABLE OF CONTENTS

LIST OF TABLES

List of Figures

1.0 CHEMISTRY, USE, AND HUMAN EXPOSURE

1.1 Chemistry

1.1.1 Nomenclature

The CAS RN for ethylene glycol is 107-21-1. Synonyms or trade names for ethylene glycol include 1,2-Dihydroxyethane; 1,2-Ethanediol; Dowtherm 4000; Dowtherm SR 1; DuPont Zonyl FSE Fluorinated Surfactants; DuPont Zonyl FSO Fluorinated Surfactants; EG; Ethane-1,2-diol; ethylene dihydrate; ethylene alcohol; Ethylene glycol; Ethylene Glycol ; Fridex; Glycol; Glycol Alcohol; lutrol-9; macrogol 400 bpc; M.E.G.; monoethylene glycol; norkool; tescol; ucar 17 *(1)*.

1.1.2 Formulas and Molecular Weight

Figure 1-1. Formulas and molecular weight (mw) of ethylene glycol.

Chemical formula=$C_2H_6O_2$.

mw= 62.07

1.1.3 Chemical and Physical Properties

Table 1-1 lists the physical and chemical properties of ethylene glycol.

Table 1-1. Physicochemical Properties of Ethylene Glycol.

Property	Value
Vapor Pressure	0.092 mm Hg @ 25 C
Melting Point	-13 C
Boiling Point	197.6 C @ 760 mm Hg
Specific Gravity	1.1088 @ 20 C
Solubility in Water	Miscible
Log K_{ow}	-1.36
Stability	Stable*
Reactivity	Reactive with acids, bases, and oxidizing materials.*

HSDB *(2)*
*Hills Brothers *(3)*

1.1.4 Technical Products and Impurities

Purity of ethylene glycol generally exceeds 99% *(4)*. Possible trace impurities of ethylene glycol include formaldehyde, ethylene oxide, and 1,4-dioxane *(3)*.

Some trade names for ethylene glycol are listed under Section 1.1.1. Past or current manufacturers of ethylene glycol include: BASF Corporation, Dow Chemical USA, Eastman

Kodak Company, Formosa Plastics Corporation USA, Hoechst Celanese Corporation, Huntsman Corporation, Shell Oil Company, Sun Company, Inc, Union Carbide Corporation, Occidental Petroleum Corporation, and PD Glycol *(2*). Quantum Chemical Corp and Texaco Chemical Company have also been identified as manufacturers of ethylene glycol *(5*).

1.2 Use and Human Exposure

1.2.1 Production

Ethylene glycol may be manufactured by one of the following methods: *(6*)

1) Oxidation of ethylene to ethylene oxide, followed by hydration;
2) Acetoxylation: Reaction of ethylene with acetic acid in the presence of a catalyst (e.g. tellurium bromide) to form mixed mono- and diacetates that are hydrolyzed to form ethylene glycol and acetic acid;
3) From carbon monoxide and hydrogen derived from coal gassification; or
4) Oxirane Process: Catalytic oxidation of ethylene to the diacetate followed by hydrolysis to ethylene glycol.

The U.S. production volume for ethylene glycol was approximately 5 billion pounds/year between 1990-1993 *(2*) and in 1995 *(7*). Approximately 400-500 million pounds of ethylene glycol/year were imported in the U.S. during 1992-1994 *(5*).

1.2.2 Use

Worldwide, approximately two-thirds of ethylene glycol use is as a chemical intermediate in the production of polyester compounds, and about one-fourth is used as an engine coolant *(8*). Ethylene glycol is found in anti-freeze formulations, coolants, windshield de-icer fluids, and brake fluids. Typical levels of ethylene glycol in common consumer products are shown in Table 1-2.

Table 1-2. Typical Levels of Ethylene Glycol in Common Consumer Products.

Product	Percent Ethylene Glycol
Automotive brake fluid (circa 1986)	85%
Automotive brake fluid (current)	<0.1%
Automotive engine coolant	50%
Windshield washer fluid	Up to 14%
Automotive wax and polish	Up to 3%
Household floor wax and polish	1.1 to 3.5%
Latex paint	2.3 to 5%
Household tub and tile cleaner	3%
Cement sealer	2.2%
Ophthalmic solution (eye drops)	10 to 28 mg/L*
Solid stick foundation (cosmetic)	ND**

* Measured in 4 samples of 15 total samples taken.

** No data; cosmetic registered in Canada with ethylene glycol as ingredient, but no quantitative measurement given.

Source: *(9*).

1.2.3 Occurrence

In 1999, the Toxic Release Inventory (TRI) reported an estimated 8.8 million pounds of ethylene glycol released to the atmosphere from U.S. manufacturing and processing facilities *(10)*. Airport deicing operations also result in ethylene glycol release to the environment. It was estimated that 58 million pounds of ethylene glycol per year are released at the 17 busiest airports in the U.S. *(11)*.

Health Canada *(9)* cited studies reporting that, following the life cycle of antifreeze from manufacture to disposal, approximately 0.87 g of ethylene glycol is released into the environment for every liter of antifreeze solution used in automobiles, and that approximately 39% of all consumed antifreeze is lost to storm sewers. In 1995, the Hazardous Substance Release and Health Effects Database (HazDat), maintained by the Agency for Toxic Substances and Disease Registry (ATSDR), reported that at least 34 National Priority List sites in the U.S. contain measurable amounts of ethylene glycol in some environmental media *(5)*.

Ethylene glycol has a low vapor pressure (0.092 mmHg at 20°C) and Henry's law constant (6.0×10^{-8} atm-m^3/mole at 25°C), *(2)* and is therefore expected to partition to the air only slightly from soil and not at all from water. Vapor phase ethylene glycol is oxidized rapidly by photochemically produced hydroxyl radicals and has an estimated half-life of about 50 hours *(2)*. The California Air Resources Board stated that no data are available for ambient levels of ethylene glycol in outdoor or non-workplace indoor air *(9)*. Health Canada also identified no sources of data on non-workplace indoor air levels of ethylene glycol, but did present some levels in outdoor air that were associated with point source emissions *(9)* (see Table 1-3). Thus, exposure through inhalation is not expected to be great for the general population.

Because it is miscible in water and highly mobile in soils, ethylene glycol that has been spilled on the ground will leach through soil into ground water or surface water, thereby producing an exposure pathway of concern *(5)*. Data on levels measured in drinking water, however, are not available, and reported levels in surface and ground water are limited mostly to areas of known contamination, particularly airports. The limited data available indicate that surface water levels of ethylene glycol are generally low (a few micrograms per liter), while wastewater from glycol production plants have averaged up to 1,300 mg/L, and runoff water samples from airports have shown the highest levels *(8)*. Ethylene glycol concentrations were measured at 19,000 mg/L in stormwater runoff at Salt Lake City International Airport and 0-100,000 mg/L at Denver's Stapleton Airport *(11)*. Table 1-3 provides examples of measured levels of ethylene glycol in surface and ground waters associated with de-icing operations at Canadian airports *(9)*. These levels are not representative of background levels likely to be found in areas unassociated with a known ethylene glycol source.

Migration rates in various soils of 4 to 27 cm per 12-hour period for ethylene glycol have been reported *(8)*. In soil and water, biodegradation is the primary means of ethylene glycol removal, with aerobic conditions effecting complete biodegradation within several days, and anaerobic conditions requiring slightly more time *(2)*. ATSDR *(5)* reported estimated half-lives of ethylene glycol in various environmental media as follows: 2–12 days in water under aerobic conditions, 8–48 days in water under anaerobic conditions, 0.3–3.5 days in the atmosphere, and 0.2–0.9 days in soil. Bioconcentration in fish is expected to be low due to ethylene glycol's low octanol-water partition coefficient (log K_{OW} = -1.36) *(5)* and low bioconcentration factor of 10 1/kg *(7)*. Therefore, exposure to ethylene glycol through ingestion of fish or other animal products is not expected to be significant.

Ethylene glycol can be found in food due to its approved uses as an indirect food additive. Polyethylene glycol, an ingredient of regenerated cellulose films (RCF) used as food wraps, can contain ethylene glycol at ≤0.2% by weight [2000 ppm] *(12)*. Ethylene glycol is also approved as an ingredient of polyethylene terephthalate (PET) the material used to manufacture soft drink bottles *(13)*. Food surveys have demonstrated the presence of ethylene glycol in food packaged in RCF and PET, as described in Section 1.2.4.

Table 1-3. Levels of Ethylene Glycol in Selected Environmental Samples.

Source	Vicinity of measurement to source	Levels measured or modeled	Reference
Outdoor Air			
Airport (Ontario)	Unknown	3200 and 4100 μg/m^3	Health Canada *(9)*
Bridge de-icing operation (Louisiana)	Unknown	<50 to 10,570 μg/m^3 (total airborne); <50 to 330 μg/m^3 (aerosol)	Health Canada *(9)*Abdelghani et al. *(14)*
Ethylene glycol manufacturing plant (Alberta)	1.8 km 4.0 km 6.8 km Surrounding prairie	100 μg/m^3 (modeled) 50 μg/m^3 (modeled) 25 μg/m^3 (modeled) 0.0012 μg/m^3 (modeled)	Health Canada *(9)*
Surface Water			
Airport (Winnipeg)	Tributary, < 2 km downstream	2 to 660 mg/L	Health Canada *(9)*
Airport (Toronto)	Tributary, <1 km downstream	<25 mg/L (detection limit)	Health Canada *(9)*
Airport (Newfoundland)	Tributary	5 mg/L (detection limit) to 80 mg/L (year 1997/1998); 5 mg/L (detection limit) to 170 mg/L (year 1998/1999)	Health Canada *(9)*
Ground Water			
Airport (Calgary)	Unknown	4 mg/L to 38 mg/L	Health Canada *(9)*
Airport (Montreal)	Unknown	8 mg/ L to 42 mg/L	Health Canada *(9)*

No data was available for indoor air and drinking water.

1.2.4 Human Exposure

1.2.4.1 General Population Exposure

The general population can be exposed to ethylene glycol through dermal contact with consumer products such as anti-freeze, coolant, windshield de-icer, or brake fluids. Accidental or intentional ingestion of ethylene glycol-containing products has been reported, and levels resulting in toxicity are discussed in Chapter 2. In the year 2000, more than 5,000 cases of ethylene glycol poisonings were reported to poison control centers in the U.S. *(15)*. Because ethylene glycol is

readily soluble in water, drinking, bathing in, or showering with contaminated water are potential exposure routes. However, there is no known information that reports levels of ethylene glycol in drinking water *(9)*. The EPA has established 70 mg/L as the drinking water equivalent level (DWEL) for ethylene glycol, which is a lifetime exposure concentration that is considered to be protective of health *(16)*.

Consumer exposure to ethylene glycol through food ingestion is possible if the food is packaged in polyethylene terephthalate (PET) bottles, which may contain unreacted ethylene glycol, or in regenerated cellulose films (RCFs), which may contain polyethylene glycol as a softening agent. Ethylene glycol was found to migrate in small amounts from 32-ounce PET bottles to a 3% acetic acid solution (a simulant for foods of pH 5.0 and below) after a 6-month storage period at 32°C, resulting in a concentration of about 100 ppb, or 94 µg ethylene glycol per bottle *(17)*. In a U.K. study to determine migration of ethylene glycol from experimental RCFs coated with polyethylene glycol, ethylene glycol was detected at levels of <10–34 ppm, in various cakes, pies, and sweets that had been wrapped in those RCFs for various lengths of time *(18)*. The results of this study are shown in Table 1-4. There is no known data that examine ethylene glycol levels in food packaged in RCF wraps that are currently approved for use in the U.S.

Table 1-4. Migration of Ethylene Glycol From Various Types of Cellulose Wraps to Various Food Types *(18)*.

Food type	Number of samples	Storage (days)	Ethylene glycol concentration in food (mg/kg)
Boiled sweet	4	168 or 450	14-34
Toffee	4	168 or 450	<10-22
Madeira cake	4	21 or 28	<10-22
Fruit cake	4	84 or 336	27-34
Meat pie	6	3 or 7	<10

Other food surveys reported by Health Canada *(9)* have found ethylene glycol in Italian wines at levels up to 6.25 mg/L (origin of contamination unknown) and in French breads preserved with ethylene oxide in airtight bags at levels up to 92.2 mg/kg. Ethylene glycol has been reported to be produced naturally in small, and presumably negligible, amounts in certain plants and edible fungi *(9)*.

Health Canada *(9)* estimated human exposures to ethylene glycol occurring through dermal contact with consumer products, dietary intake, and inhalation of air and ingestion of soil near point sources. Health Canada noted that the available exposure data were very limited. CERHR agrees that the exposure data are limited. For example food estimates were based on levels of ethylene glycol measured in foods packaged in an experimental RCF manufactured in the UK and not a market basket survey. Due to the limitations in data, Health Canada used conservative assumptions in their estimates, as is typical for a regulatory agency. Despite the use of conservative assumptions that are likely to overestimate actual human exposures, the estimates were very low, in the µg/kg bw/day range.

1.2.4.2 Occupational Exposure

Occupational exposure to ethylene glycol may occur through dermal contact while handling products containing this compound, or through inhalation of airborne ethylene glycol that results from heating or spraying processes *(5)*. Ethylene glycol releases can occur during the manufacturing of polyethylene terephthalate and other synthetic organic chemicals *(2)*. Ethylene glycol is also present in industrial adhesives as well as paint, primer, and varnish formulations; *(2)* thus, workers who manufacture or use such formulations may be potentially exposed either dermally or through inhalation of the volatilized compound.

A 1981−1983 National Occupational Exposure Survey (NOES) survey of U.S. workers led NIOSH to estimate that 1,133,792 people (352,752 of which were female) were potentially exposed to ethylene glycol at the workplace *(2)*. In a study of workers spraying ethylene glycol from trucks onto bridge surfaces for de-icing purposes, personal air sampling inside the trucks recorded concentrations of <0.5–3.36 mg/m^3 ethylene glycol vapor and <0.05–2.33 mg/m^3 ethylene glycol aerosol *(14)*. De-icing operations on airport runways are another important exposure scenario for workers. Health Canada *(9)* cited studies reporting that 50% of the glycols used in aircraft de-icing falls to the ground in the vicinity of the airplane, while 16% remains on the airplane and 35% is blown off by wind.

Gerin et al. *(19)* conducted a study to measure ethylene glycol exposure and kidney function in 33 Canadian aviation workers exposed to ethylene glycol de-icing fluid. The discussion of kidney function is included in Section 2. The de-icing fluid (Union Carbide's UCAR D) contained about 45% ethylene glycol, 5% diethylene glycol, and 50% water plus other additives. Before spraying, the fluid was diluted with 50-70% water and heated to 70–80°C. The study was conducted in Quebec from January to March in 1992. Table 1-5 outlines concentrations of ethylene glycol measured in the breathing zones and urine of workers. A total of 154 ethylene glycol vapor and mist samples were taken. Because the values were not weighted for exposure time, exposure durations for detected concentrations are listed in Table 1-5. Eighteen vapor samples exceeded the quantification limit (2.5 mg/m^3), but only 3 samples had quantifiable levels of ethylene glycol mists (≥17 mg/m^3). Urine samples were obtained prior to, immediately after, and the morning after the shift. Thirty-three to forty-two urine samples were collected for each period. A threshold value of 5 mmol/mol creatinine was selected because levels below that limit were thought to be unrelated to occupational exposure. A total of 16 urine samples had ethylene glycol concentrations that exceeded the threshold value. Diethylene glycol was found in some air and urine samples at about one-tenth the level of ethylene glycol. The authors concluded that the highest exposures to ethylene glycol occurred in basket operators and coordinators who were most likely to have contact with the greatest concentrations or to be accidentally sprayed. Most basket operators wore masks that offered some protection against mists but not vapors. Because ethylene glycol was not detected in air samples of some workers with the highest urine values, the authors suggested that the workers could also be exposed through oral intake and dermal contact. **[As discussed in greater detail in Section 2.1.1.1.2, the Expert Panel noted that appropriate air sampling methods were used in this study. Personal breathing samples were obtained to measure both vapors and mists. The ability to examine the statistical correlation between air and urinary ethylene glycol levels was limited by the small number of air and urine samples with values exceeding their respective limit of quantification and threshold values. The limited amount of data do not allow firm conclusions to be made regarding the most significant exposure route(s). The study does demonstrate that deicing operations can result in ethylene glycol mist exposures greater than the ACGIH limit of 100 mg/m^3 in workers, with exposures possibly occurring through multiple routes.]**

Table 1-5. Ethylene Glycol in Air Samples and Urine of Aviation Workers.

Job (Number of air samples)	Vapor Level; n=number samples≥LQ (Duration of exposure)	Mist Level, n=number samples≥LQ (Duration of exposure)[a]	Urine Concentrations (mmol/mol creatinine), n=number samples≥TV[a,b]		
			Pre-Shift	Post-Shift	Next Morning
Lead (n=25)	5 mg/m^3; n=1 (25 minutes) [a]	<17 mg/m^3; n=0	<5; n=0	<5; n=0	<5; n=0
Truck Driver (n=27)	2.5, 3.4 mg/m^3; n=2 (43, 62 minutes) [a]	<17 mg/m^3; n=0	<5; n=0	6.8; n=1	6.4; n=1
Coordinator (n=18)	7.3 mg/m^3; n=1 (15 minutes) [a]	91 mg/m^3; n=1 (118 minutes)	<5; n=0	129; n=1	11.6, 13.6, 14.8; n=3
Basket Operator (n=84)	0.9-22.0 mg/m^3; n=14 (117-118 minutes)	76, 190 mg/m^3; n=2 (45, 75 minutes)	<5; n=0	5.2, 5.6, 6.2, 7.6, 10.4, 10.6, 11.8, 15.9; n=8	7.7, 14.5; n=2

LQ=Limit of Quantification=2.5 mg/m^3 for vapor5 and 17 mg/m^3 for mist
TV=threshold value for urine= 5 mmol/mol creatinine
[a]Values for individual workers.
[b]A total 16-21 urine samples/time period were taken from basket operators; 5-7 urine samples/time period were measured in each of the other categories.

Laitinen et al. *(20)* examined exposure to ethylene and propylene glycol in Finnish motor servicing workers using the method of Tucker and Deye *(21)*. Ten male mechanics from 5 different garages participated in the study. The only protective equipment used by some workers was leather gloves. Ten age-matched male office workers served as controls. Differences between groups were evaluated by Student's t-test. Air concentrations of ethylene glycol and propylene glycol were measured during the entire shift. Neither ethylene glycol nor propylene glycol vapors were detected in the breathing zones of workers; detection limits for each compound were 1.9 cm^3/m^3 and 3.2 cm^3/m^3, respectively **[cm^3/m^3 equivalent to ppm]**. Urine samples were collected after the work shift and analyzed for ethylene glycol, oxalic acid, and propylene glycol. Possible biochemical indicators of toxicity were also analyzed in urine and are discussed in Chapter 2. Urinary concentrations of ethylene glycol were significantly higher in mechanics versus controls (7.3±4.7 vs. 1.7±0.7 mmol/mol creatinine, respectively). Levels of oxalic acid were also higher in mechanics, but statistical significance was not achieved (47±11 vs. 36±14 mmol/mol creatinine). Propylene glycol concentrations were not increased in urine from mechanics. The study authors noted that ethylene glycol excretion was higher in workers who conducted major engine repairs and were exposed to ethylene glycol for longer time periods. Because ethylene glycol was not detected in air, but was detected in the urine of workers, the study authors concluded that exposure occurred through dermal contact. **[As discussed in greater detail in Section 2.1.1.1.2, the Expert Panel noted that only exposures to vapors, and not mists, were measured. Because only vapors were measured, it is not known if mists were**

present possibly due to aerosol generation while handling the fluid or due to temperature-related condensation. Ethylene glycol exposure would be underestimated if mists were present. However, mist exposure during automotive maintenance would seem less likely than during aircraft deicing operations. Given this caveat, the study data are suggestive that dermal contact may be a route of exposure for this occupation.]

Abdelghani et al. *(14)* measured personal time weighted average (TWA) exposures to ethylene glycol vapors and mists in 8 workers who were de-icing bridges with a 50% ethylene glycol solution sprayed from a truck over a period of 6 to 9 hours. Sampling was conducted on two separate dates to obtain a total of 16 samples. During sampling, the window on the driver side of the truck was closed while the passenger side window was open. During normal operations, both windows are usually closed. TWA exposures to ethylene glycol mists and vapors were measured at <0.05-0.33 mg/m^3 and <0.05-10.38 mg/m^3. Fifteen minute ceiling values were measured at <0.05–2.33 mg/m^3 for aerosols and <0.05–3.36 mg/m^3 for mists.

A ceiling limit of 50 ppm (125 mg/m^3), established for ethylene glycol by the Occupational Safety and Health Administration (OSHA), was vacated in 1989, although it is still enforced in some states *(2)*. The National Institute for Occupational Safety and Health (NIOSH) has questioned whether this level is adequate to protect workers from recognized health hazards *(2)*. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a ceiling exposure limit of 100 mg/m^3 for ethylene glycol aerosols, to prevent respiratory and occular irritation *(22)*.

1.3 Utility of Data

Limited exposure data for ethylene glycol was available for review by the Expert Panel. The utility of occupational exposure data is limited by either small sample size or a high proportion of non-detected values. Estimates of ethylene glycol-exposed workers are based on a 1981-1983 National Occupational Exposure Survey which is approximately 20 years old and may not accurately reflect the number of currently exposed workers. The applicability of reported ethylene glycol levels in food for consumer exposure is also unclear due to differences in packaging materials and the limited number of foods tested.

1.4 Summary of Human Exposure Data

Ethylene glycol is used as an engine coolant, in the manufacture of polyester, and is found in antifreeze solutions, windshield deicers, brake fluids, automotive and household waxes and polishes, latex paint, and possibly ophthalmic solutions, and cosmetics *(8, 9)*. Ethylene glycol is approved as an indirect food additive. It is used to manufacture polyethylene glycol, an ingredient of regenerated cellulose film (RCF) used as food wraps *(12)*. Ethylene glycol is also an approved material for polyethylene terephthalate (PET), the material used to manufacture soft drink bottles *(13)*. Between 1990-1993, five billion pounds of ethylene glycol were produced in the United States per year *(2)*. Significant amounts of ethylene glycol are released to the atmosphere. In 1999, 8.8 million pounds of ethylene glycol were released into the environment by U.S. manufacturing and processing facilities *(10)*. Airplane deicing operations result in the release of an estimated 58 million pounds of ethylene glycol per year at the 17 busiest airports in the U.S. *(11)*. For every liter of automobile antifreeze solution used, it is estimated that 0.87 g of ethylene glycol is released to the environment *(9)*.

Exposure to ethylene glycol by the general public can possibly occur from dermal contact with products such as antifreeze solutions, ingestion of food or beverages containing trace amounts of ethylene glycol leaching from packaging materials, and inhalation of air and ingestion of soil near point source emissions. Due to its low vapor pressure, very little ethylene glycol is expected to be present in outdoor air, with the possible exception of point source emissions. Therefore, significant exposure through outdoor air is not expected for the majority of the general population. Drinking or bathing in water is a potential source of exposure, but there is no information about ethylene glycol levels in water. Health Canada *(9*) estimated human exposures resulting from dermal contact with consumer products, dietary exposure, and inhalation of air and ingestion of soil near point sources. Because the human exposure data are limited, Health Canada used very conservative assumptions in their estimates. Although the values likely overestimated actual human exposure levels, the estimated exposure to ethylene glycol was very low, in the μg/kg bw/day range.

Occupational exposure to ethylene glycol can occur during its use as a chemical intermediate and as an ingredient of automotive care products, deicing solutions and adhesives and paints. Exposure of workers is most likely to occur from dermal contact of ethylene glycol containing solutions and inhalation of airborne vapors and mists generated through heating and spraying processes. However, exposures in workers are not well characterized. In a study of bridge deicing workers, Abdelghani et al. *(14*) measured personal TWA ethylene glycol exposures of <0.05-0.33 mg/ m^3 mists and <0.05-10.38 mg/ m^3 vapors; fifteen-minute ceiling values ranged from <0.05-2.33 mg/ m^3 mists and <0.05-3.36 mg/m^3 mists. A study of airport personnel measured personal air exposures ranging from <17-190 mg/ m^3 mists and <2.5-22 mg/m^3 vapors, *(19*) urinary concentrations of ethylene glycol were found to be increased in some workers compared to non-occupational levels, but correlations between personal breathing samples and urinary levels of ethylene glycol were not conducted due to limited data above limits of quantitation. A study of mechanics found increased urinary ethylene glycol levels compared to unexposed workers *(20*). Though ethylene glycol vapor levels were below the detection limit in area air samples, the Panel noted that mist levels were not measured. Limitations in the occupational exposure studies *(19, 20*) do not allow a determination to be made about the most significant exposure route in workers. ACGIH *(22*) recommends a workplace ceiling exposure limit of 100 mg/m^3 for ethylene glycol aerosols, based on respiratory and ocular irritation.

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS

2.1 Toxicokinetics and Metabolism

The first step in the evaluation of toxicokinetics and metabolism data for ethylene glycol was an examination of authoritative reviews (i.e., reviews by agencies such as ATSDR *(5*) and NTP *(23*) to determine what is known as a function of route, dose, and species. Those reviews were summarized, compared, and contrasted to communicate facts about toxicokinetics and metabolism. Reviews written by independent authors *(24*) and *(25*) were also examined and it was noted if conclusions were consistent or in contrast to those reached by authoritative sources. There were cases where it was found necessary or beneficial to thoroughly summarize and evaluate original studies. Such cases included important studies in interpreting developmental or reproductive toxicity issues, significant studies not included in reviews, and studies that clarified previous uncertainties. A limited number of studies in non-rodent species were also reviewed in detail to allow for comparison of interspecies variability. In reviewing key original studies, the Expert Panel prepared statements on the strengths, weaknesses, and utility of the studies.

As described in the developmental toxicity section of this document (See Section 3) mice are more sensitive than rats to ethylene glycol-induced developmental toxicity. Compared to rodents, rabbits are more susceptible to maternal toxicity but less sensitive to adverse developmental outcomes. Mice are less sensitive to ethylene glycol-induced developmental toxicity when administered dermally versus orally). These variations in toxicity among different species and through different exposure routes may be due to variations in toxicokinetics. Therefore, the focus in this section will be on studies that may provide insight on these issues.

2.1.1 Absorption

2.1.1.1 Human

2.1.1.1.1 Oral

Ethylene glycol is readily absorbed in humans following oral intake as evident by high levels in serum and rapid onset of clinical symptoms *(5, 24*). A limited number of studies reported ethylene glycol blood levels in humans, mostly after acute poisonings. Those levels were reported to range from 14.5 to 650 mg/dL **[2.3-105 mM]** *(5*). Since most of those values were measured a number of hours following exposure, they may be less than peak concentrations.

2.1.1.1.2 Inhalation

There is no quantitative information about absorption of ethylene glycol in humans following inhalation exposure; indirect evidence of absorption can be assessed through a controlled study *(26*) and two occupational exposure studies *(19, 20*). In a study conducted by Wills et al., *(26*) men exposed to 17-49 mg/m^3 ethylene glycol aerosols for 30 days experienced no increase in serum or urine levels of ethylene glycol compared to controls. Complete details of this study are included in Section 2.2.1. The study authors stated that the study suggests poor absorption of ethylene glycol through the respiratory tract of humans. **[The Expert Panel questioned this conclusion. They noted that the lowest amount of glycol ethylene detectable in urine via the study author's analytical method was reported to be 7 mg/100 mL (detectable peak) or 10mg/100 mL (bottom of standard curve). However, 7.7 mg/100 mL was reported as the maximum urine concentration in the exposed group. It would appear that the method detection limit was in the concentration range that these individuals had in urine and thus**

was not sensitive enough to reliably detect differences between exposed and control workers. Further, at the relatively low doses of ethylene glycol administered, it is possible that the majority of the dose was excreted in exhaled breath as CO_2 plus some as metabolites in urine. Thus, the lack of detection of elevated ethylene glycol in urine cannot be taken as evidence of low percent absorption. The lack of ethylene glycol elevation in serum relative to unexposed controls might also be due to analytical difficulties. While the method detection limit was not reported, the authors state that a step for the removal of carbohydrates from serum had not been incorporated into the method; they state that this led to higher and more variable ethylene glycol results than would otherwise be obtained. This may have obscured any exposure/control differences in ethylene glycol serum levels.]

In a Finnish study, *(20*) ethylene glycol levels in urine were higher in mechanics compared to unexposed controls even though levels of ethylene glycol vapors in the breathing zones of mechanics were below the detection limit. The finding led the study authors to conclude that exposure occurred through dermal contact. **[The Expert Panel noted that the air sampling method used in this study would likely capture only ethylene glycol vapors and not mists. Evaluation of the extent of inhalation exposure to ethylene glycol would be underestimated if significant mist levels were present in the worker's breathing zones. Mist could be present due to aerosol generation while working with the fluid or due to condensation of ethylene glycol at ambient temperatures in the garage. However, mist exposures during car maintenance, would seem much less likely than during aircraft deicing operations. Given this caveat, the study data are suggestive that dermal contact may be a route of exposure for this occupation.]**

A study of Canadian aviation workers found that some workers who were not exposed to detectable levels of ethylene glycol in air had the highest levels of ethylene glycol in urine; study authors speculated that exposure could have occurred through oral intake and dermal contact *(19*). **[This study involved appropriate sampling methods (personal breathing zone sampler; sampling train to capture both vapor and aerosol) to evaluate worker exposure to ethylene glycol from airplane deicing procedures. The exposure data were highly variable. Only 8 of the 22 most likely exposed workers (basket operators) had urinary ethylene glycol levels above baseline levels found in non-exposed subjects. The ability to examine the statisticaal correlation between air and urinary ethylene glycol levels was limited by the small number of air and urine samples with values exceeding their respective limit of quantification and threshold values. The only support for the author's speculation that inhalation exposure is not the only route of exposure is a statement in the discussion that several workers excreted elevated levels of ethylene glycol in urine and did not have detectable levels of ethylene glycol in their air samples. For several workers, dermal and even ingestion exposure may have contributed to urinary ethylene glycol levels; however, the data in this study are too limited to draw firm conclusions regarding the importance of any single dose route in this industry. What can be said is that this paper demonstrates that under certain conditions, airplane deicing crews can be exposed to ethylene glycol mists greater than 100 mg/m^3 with a variety of dose routes (inhalation, dermal, oral) possibly contributing to urinary ethylene glycol levels above non-occupational exposures.]**

2.1.1.1.3 Dermal

ATSDR *(5*) describes two *in vitro* skin absorption studies conducted with cadaver skin by Loden *(27*) and Driver et al. *(28*). In those two studies, average skin absorption rates were found to be quite variable between the two studies and ranged between 0.09 and 118 $\mu g/cm^2/hr$.

An additional study by Sun et al. *(29*) was identified and found to be the most comprehensive study since *in vitro* dermal absorption rates of undiluted and 50% [^{14}C]-ethylene glycol (97% purity) were compared in human and mouse skin Fresh, full-thickness skin samples were obtained from the abdomens of 5-6 female volunteers (age 20-60 years old) and the dorsal trunks of 3 female Crl: CD-1 mice (8-weeks-old). The entire skin surfaces were covered with an "infinite dose" of ethylene glycol (22-28 mg/cm^2) and incubated for 6 hours in covered cells containing minimum essential medium as the receptor fluid. At the end of the incubation period, radioactivity in receptor fluid, skin, and skin wash was measured by liquid scintillation spectrometry. [^{14}C]-ethanol was used as a reference chemical to assess integrity of skin samples and it was verified that skin samples were normal since permeability rates of [^{14}C]-ethanol were within historical ranges. Results of the study are listed in Table 2-1. As noted in Table 2-1, the lag times to steady state were 3 times longer in human compared to mouse skin. Steady state penetration rates and permeability constants were 30-40 times less in human compared to mouse skin for both undiluted and 50% ethylene glycol. Within both species, the permeability constant for undiluted versus 50% ethylene glycol were approximately the same, while steady state penetration rates were twice as high for undiluted versus 50% ethylene glycol. In a comparison of results to those of other laboratories, the authors noted their penetration rate was much lower than that obtained by Loden, *(27*) who used frozen skin. The authors speculated that deterioration may occur during storage and thawing of skin samples thus reducing barrier properties. Authors also noted that their penetration rates were much higher than those obtained by Driver et al. *(28*) and they speculated that the low dose (8 µg/cm^2) used by Driver et al. does not represent an "infinite dose." In closing, the authors concluded that human skin is significantly less permeable to ethylene glycol than mouse skin.

Strengths/Weaknesses: Details of the Sun et al. *(29*) study are generally well reported. However, there is a key inconsistency in the data that may question the weight one would want to place on the results. The mouse and human skin preparations were evaluated for integrity by first testing them against radiolabeled ethanol, an agent with known *in vitro* penetrant rates in both species. These results found Kp values to be essentially the same across the mouse and human skin specimens, with the ethanol Kp results being 2 to 3 times faster than the ethylene glycol results in mouse skin. This would appear to make sense as adding an extra polar group onto ethanol might be expected to retard ethylene glycol passage across the various dermal layers. However, the human skin specimens (n=5 to 6) had Kp values that were 30 to 40 fold below the mouse values suggesting that the permeability of ethylene glycol in human skin is not only far below its permeability across mouse skin, but also far below the permeability of ethanol across the same human skin specimens. While this could certainly have occurred, the fact that such a large ethanol:ethylene glycol Kp differential was not seen in mouse skin raises questions about why human skin should show such a large discrepancy between these related chemicals. The study authors focused on the mouse:human difference in ethylene glycol Kp and did not discuss or even acknowledge this curious ethanol:ethylene glycol Kp difference in human but not mouse skin.

Utility (Adequacy) for CERHR Evaluation Process: The utility of the Sun et al. *(29*) study is questionable without further investigation. Given that there is a wide variability in ethylene glycol human skin permeability results across the three available dermal absorption studies, *(29, 28, 27*) it would seem prudent that risk assessment on dermal exposure to ethylene glycol include dermal absorption equations and factors presented in USEPA guidance documents. These equations enable a modeling-based approach for deriving Kp across human skin which is informed by structure activity relationships (SAR) for dermal penetrability. This approach could then be used to help decide which of the reported ethylene glycol Kp values appears to be most consistent with dermal penetration principles and with results for other chemicals. This being

said, it is clear from the Frantz et al. *(30, 31, 32*) series of publications in rats that dermal exposure will yield plasma concentrations of ethylene glycol and metabolites far below an equivalent oral (bolus) dose. Thus, one does not have to invoke an assumption of much slower dermal penetration in humans than rodents to still come to the conclusion that human dermal exposure is unlikely to result in acute poisoning, unless perhaps there is an extreme exposure scenario, or if the skin barrier function has been seriously compromised.

Table 2-1. Results of Skin Absorption Study by Sun et al. *(29*).

Parameter	Mouse		Human	
	Undiluted Ethylene Glycol	50% Ethylene Glycol	Undiluted Ethylene Glycol	50% Ethylene Glycol
Lag Time to Steady State (hours)	1.02	0.90	3.07	3.10
Steady State Penetration Rate (mg/cm^2/hour)	0.52	0.22	0.013	0.007
Permeability Constant (cm/hour x 10^{-4})	4.68	4.36	0.12	0.14
% Cumulative Absorbed Dose	10.82	4.41	0.14	0.08
% Total dose Recovery	91.91	75.00	76.48	88.72

2.1.1.2 Animals

2.1.1.2.1 Oral

Studies in rats, mice, dogs, rabbits, and monkeys consistently demonstrated that absorption of ethylene glycol administered by gavage is fast and nearly complete. Gavage administration of a high ethylene glycol dose (~1000 mg/kg bw) resulted in maximum blood levels of ethylene glycol at 0.2-0.6 hours in mice *(31, 32*), ~1 hour in rats *(30, 32, 33*), and rabbits, *(34*) 1-2 hours in monkeys, *(35*) and 2 hours in dogs *(36*). Pottenger et al. *(33*) demonstrated that the absorption rate of ethylene glycol does not differ between non-pregnant and pregnant rats on gestation day (gd) 10. Carney *(24*) noted that blood levels of ethylene glycol increase linearly according to the oral dose administered and are very similar among different species. Table 2-2 outlines blood levels of ethylene glycol in various studies.

Table 2-2. Maximum Levels of Ethylene Glycol in Blood Following Gavage Exposure to Ethylene Glycol.

Sex and Species	Dose (mg/kg bw)*	Blood Ethylene Glycol Level (mM)	Reference
Male and Female Sprague Dawley rats.	10 1,000	0.2 21	Frantz et al. *(30, 32)*
Female Sprague Dawley rats.	10 2,500	0.15 45.0	Pottenger et al. *(33)*
Pregnant Female Sprague Dawley rats.	10 150 500 1,000 2,500	0.13 1.4 6.31 14.3 56.8	Pottenger et al. *(33)*
Male Sprague Dawley rats.	2,000	31	Hewlett et al. *(36)*
Female CD-1 mice.	10 100 200 400 1,000	0.1 1.6 4.7 7.3 16.4	Frantz et al. *(32)*
Male and female rhesus monkeys.	1,109	20.1	McChesney et al. *(35)*
Male and female mixed-breed dogs.	1,000-1,036	29	Hewlett et al. *(36)*

*All doses except those reported by Hewlett et al. *(36)* were converted from original units by CERHR. Example calculation for pregnant rats exposed to 2,500 mg/kg bw ethylene glycol in Pottenger et al. *(33)* study: 3528 µg ethylene glycol/g blood x ~1 g blood/1 mL blood x 1000 mL/L x 1 mg/1000 µg x 1 mmol ethylene glycol/62.07 mg = 56.8 mM.

In contrast to rapid and nearly complete absorption following oral exposure, absorption through the skin and lung appear to be slow and incomplete. A dermal study in rats and mice by Frantz et al. *(30, 31, 32)* and an inhalation study in rats by Marshall and Cheng *(37)* were reviewed in detail, since there are so few data by these exposure routes and absorption data is not as well characterized as the oral exposure data.

2.1.1.2.2 Dermal

Frantz et al. *(30, 31, 32)* reported that dermal application of a neat (10-1,000 mg/kg bw) or 50% aqueous solution (1000 mg/kg bw) of ethylene glycol results in slow and incomplete absorption. In two mass balance studies, absorption of ethylene glycol was determined by measurement of radioactivity in body tissues, exhaled air, and excreta. For male and female rats, approximately 32, 29-36, and 22-26% of the 10 mg/kg bw and 1,000 mg/kg bw doses and the 50% solution were absorbed, respectively *(30, 31)*. The respective percentages absorbed in mice treated with 100 and 1000 mg/kg bw and 50% solution were 43, 51, and 39% *(31)*. The authors concluded that absorption of undiluted and 50% ethylene glycol was greater in mice versus rats. Authors noted that the half-life for dermal absorption was about an order of magnitude longer than the half-life for oral absorption. Additional details of these studies and a complete Expert Panel evaluation is included under the Metabolism and Excretion Section of this report.

2.1.1.2.3 Inhalation

Marshall and Cheng *(37)* evaluated the deposition and fate of inhaled ethylene glycol vapor and condensation aerosol in Fischer 344 rats. Two groups of 15 male and female Fischer 344 rats/sex

(13-17-weeks-old) were exposed to ^{14}C-ethylene glycol (>99% purity) by nose only in the form of vapors (actual concentration=32 mg/m^3) for 30 minutes or aerosols (actual concentration=184 mg/m^3; MMAD=2.3 μm) on Ga_2O_3 particles for 17 minutes. **[The Expert Panel converted the inhalation doses to mg/kg bw/day values by using the minute volume of rats reported by study authors (1.3 mL/min/g bw). Exposure to vapor was estimated to be 1.25 mg/kg bw (32 mg/m^3 * 0.47 m^3 inhaled/24 hours * 0.5 hours * 1/0.25 kg, assuming 100% absorption). Exposure to aerosol was estimated at 7.1 mg/kg bw by using the same equation described above.]** The aerosol dose was based on human studies by Wills et al. *(26*) that demonstrated humans could tolerate an ethylene glycol atmosphere of 188 mg/m^3 for 15 minutes. The vapor concentrations were based on previous observations that 20% of total glycol is present as vapor when aerosols are generated. Deposition of ethylene glycol was determined by measuring radioactivity in different regions of the respiratory tract and other body tissues at intermittent times from 10 minutes to 6 days following exposure. Rats exposed to vapor had an initial body burden of 0.74 mg/kg bw ethylene glycol, while rats exposed to aerosol had an initial body burden of 2.4 mg/kg bw. Approximately 60% of the vapor or aerosol inhaled were deposited, largely in the nasal cavity. Between 75 and 80% of the initial body burden was found throughout the body, indicating rapid absorption and distribution following deposition in the nasal cavity. Excretion patterns observed in this study and an Expert Panel critique of this study are discussed under Section 2.1.3.2.

2.1.2 Distribution

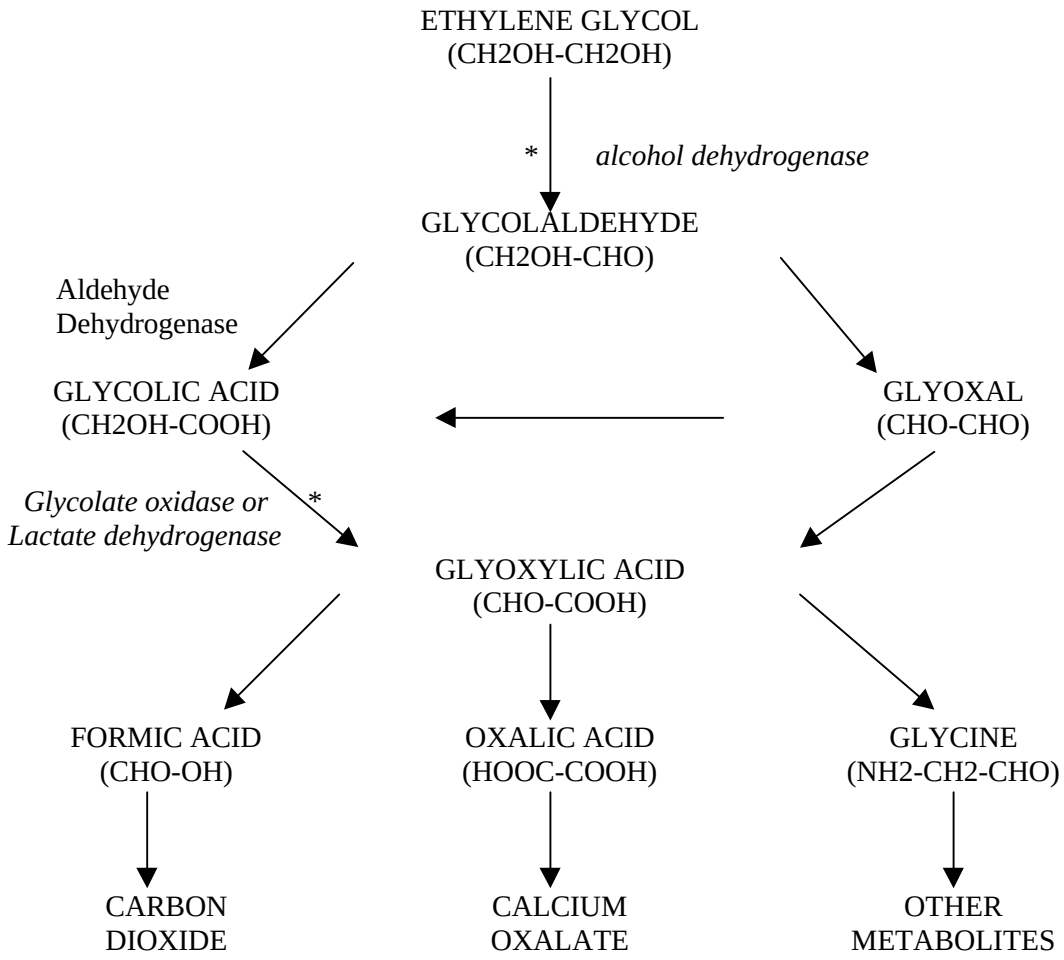
Analyses of tissue, plasma, and urine deposition of ethylene glycol in humans, rats, mice, monkeys, and dogs, indicate that it is readily distributed according to total body water *(5, 24*). The volume of distribution in two patients was estimated to be 0.54-0.56 L/kg bw and the urine to plasma ratio in one patient was estimated at 1.0-1.4 *(5*).

2.1.3 Metabolism

Metabolism of ethylene glycol has been discussed in reviews by ATSDR, *(5*) Carney, *(24*) NTP, *(23*) and Weiner and Richardson *(25*). The reviews provide generally consistent descriptions of the metabolic process. Figure 2-1 outlines the metabolic pathway of ethylene glycol, which is qualitatively similar in humans, monkeys, dogs, rabbits, rats, and mice. Initially, ethylene glycol is converted to glycolaldehyde by nicotinamide adenine dinucleotide (NAD)-dependent alcohol dehydrogenase (ADH) in a rate-limiting reaction. Reduction of NAD leads to concomitant formation of lactate from pyruvate. **[However, the Expert Panel noted that knowledge regarding the specific ADH enzymes involved remains deficient. The data to date suggest a major role for the class I alcohol dehydrogenase; however, studies to date have not addressed which of the three class I ADH enzymes is most important. The class I ADH locus encodes three enzymes that can homo- or heterodimerize to form the active enzyme, i.e., ADH1A (ADH1 or ADHα), ADH1B (ADH2 or ADHβ) and ADH1C (ADH3 or ADHγ). Further, given the approaches used to implicate ADH, it is difficult to rule-out a possible role for CYP2E1. Thus, both pyrazole and 4-methylpyrazole will inhibit both ADH and CYP2E1. Studies by Cederbaum and colleagues *(38*) provided evidence that CYP2E1-generated peroxide could facilitate the non-enzymatic oxidation of ethylene glycol to formaldehyde, but did not address the question of whether or not CYP2E1 could directly participate in the oxidation of ethylene glycol to glycolaldehyde].** Glycolaldehyde is rapidly converted to glycolate and to a minor extent glyoxal by cytosolic aldehyde oxidase and aldehyde dehydrogenase. Because glycolaldehyde is rapidly metabolized, very little is found in plasma. Glycolate is a major metabolite in species examined such as humans, and its potential to accumulate (with a resultant acidosis) is of toxicological significance.

The next major metabolic step is oxidation of glycolate to glyoxylate by glycolate oxidase or lactate dehydrogenase, which is rate limiting; glyoxal is also converted to glyoxylate. Once formed, glyoxylate is primarily metabolized to formate and then respiratory CO_2 and to a lesser extent, to urinary oxalate and glycine. The potential for oxalate to form calcium oxalate crystals is toxicologically significant. As discussed in greater detail below, the major elimination products in rats, rabbits, dogs, and monkeys are carbon dioxide in expired air and glycolate and unchanged ethylene glycol in the urine. Species vary in the amount of other metabolites excreted in the urine, including glyoxylic acid, hippurate, and oxalate. Also discussed in greater detail below, is a study that found no significant differences in metabolism of ethylene glycol in pregnant versus non-pregnant rats.

Figure 2-1. Metabolism of Ethylene Glycol.



*Rate-Limiting Steps
Adapted from Carney *(24)*

2.1.3.1 Humans

ATSDR *(5)* notes that case studies of ethylene glycol poisonings in humans reported increased levels of glycolate and lactate in plasma, which were attributed to be the cause of acidosis.

Glycolate is a metabolite of ethylene glycol while lactate is generated from pyruvate during the reduction of NAD. Plasma glycolate levels of 12.2 to 29.3 mM were reported following human poisonings. The detection of calcium oxylate crystals in urine was also reported in case studies *(5*).

The detection of glycolate anion in several human poisoning cases is important in demonstrating that there is a significant potential for humans to form and retain glycolic acid, similar to what is seen in rodents. For example, Jacobsen et al. *(39*) provide plasma ethylene glycol and glycolate concentrations from 6 adult male subjects who were admitted to the hospital several hours after ingesting an unspecified quantity of antifreeze on the misconception that it was alcohol. Five of the six subjects were described as chronic alcoholics. All six were treated with ethanol and bicarbonate upon admission to prevent further metabolism of ethylene glycol and to offset the metabolic acidosis. Further, hemodialysis was begun at apparently 4 hours after admission in an effort to remove glycolate.

Data at admission on these six patients indicates a range of glycolate to ethylene glycol plasma concentration ratios: in 2 subjects the ratio was below 1 (0.62 in both cases); 1 subject it was close to unity (1.13) and in the remaining 3 it was substantially above unity (2.45, 2.95, 4.25). Some of this variability could be due to different times elapsed between ingestion and the sampling of blood as subjects were admitted sometime between 10 and 48 hours post ingestion across the 6 cases. The pre-dialysis elimination of glycolate from plasma was very slow in two patients in which serial sampling was performed for kinetic analysis. These glycolate kinetics are not affected by continued formation from ethylene glycol as ethanol had been administered to block further conversion. The plasma concentration of glycolate in the 6 subjects ranged from 17-29.3 mM upon admission, which is 24 to 41 times higher than the reported Km suggesting saturation of glycolate elimination. The acute renal failure seen in each of these patients also likely contributed to the prolonged glycolate retention.

The other case report of ethylene glycol poisoning that involved measurement of glycolate in plasma involved 3 subjects *(40*). The first was a 2 year old girl admitted to the hospital 1-1.5 hours after ingesting an unknown amount of antifreeze. The second was a 46 year old who drank a glassful of antifreeze approximately 6-8 hrs prior to admission, while the 3rd was a 14 month old girl who ingested an unknown quantity of a car wash liquid containing 1% ethylene glycol monobutyl ether. Case 1 showed elevated plasma glycolate (12.2 mM) and ethylene glycol (22.9 mM) for a glycolate:ethylene glycol ratio of 0.53 upon admission. In contrast to the data described above in adults, this elevated concentration of glycolate in a 2 year old was associated with a readily detectable elimination from plasma ($t_{1/2}$ not reported but apparently in the range of 2 to 4 hrs from their Figure 2). In contrast, the single adult case in this report had a plasma glycolate concentration of 15.4 mM upon admission which failed to substantially decrease over the first 8 hours in the hospital but then was rapidly decreased by hemodialysis. In this individual, plasma glycolate was well above ethylene glycol at admission (ratio of 4.97). The third case involved too little ethylene glycol exposure to produce symptoms or yield measurable glycolate levels in plasma.

These two clinical reports suggest that glycolic acid is a key metabolite whose levels in plasma can readily surpass those of the parent compound in poisoning cases; the reported cases have generally involved glycolate accumulation to levels that exceed metabolic detoxification capability. In cases involving renal failure, glycolate levels in blood may not appreciably drop without hemodialysis. The 2 year old girl whose serum glycolate levels dropped without dialysis was not reported to have renal failure, *(40*) in contrast to the other cases described above. It is also interesting that in the 2 year old girl, the glycolate:ethylene glycol ratio remained below

unity for the entire time course; this is quite different from what occurred in adults in which saturation of glycolate removal and renal failure maintained high glycolate levels while ethylene glycol metabolism continues (unless ethanol is used as a therapeutic intervention) thus shifting the plasma ratio towards increasingly more glycolate relative to ethylene glycol *(39*). Based upon this single pediatric cases it is impossible to determine whether children are more resistant to ethylene glycol-induced renal failure or can more readily metabolize glycolic acid.

The human data are consistent with the rat data *(33*) which indicate that ethylene glycol concentrations are above glycolate concentration in blood shortly after dosing but that this reverses as the metabolite accumulates while parent compound diminishes, a trend that is more evident at the higher ethylene glycol doses used. Thus, the in vivo data suggest an ability to form glycolate in humans that is similar to that seen in rats.

Strengths/Weaknesses: The human data, while limited, provide data potentially useful for physiologically-based toxicokinetic (PBTK) modeling of ethylene glycol metabolism and elimination in humans. The major limitation would be in specifying the amount of ethylene glycol ingested except in the one case in which an estimate could be made from the description that a glassful of antifreeze was ingested approximately 6-8 hours prior to admission *(40*). In the other cases, estimates would have to be made of quantity ingested and amount of time elapsed prior to blood sampling to fit the initial blood ethylene glycol concentration from which the remaining metabolism and elimination steps could be modeled.

Utility (Adequacy) for CERHR Evaluation Process: The human data have utility in demonstrating human metabolites associated with ethylene glycol poisoning and verifying that metabolites are consistent to those observed in animal studies.

2.1.3.2 Animals

Frantz et al. *(30, 31, 32*) examined dose-related shifts in metabolism of ethylene glycol in 10-11-week-old male and female Sprague-Dawley rats and 5-6 week-old female CD-1 mice. ^{14}C-ethylene glycol (98-99% purity) was administered by intravenous infusion in saline, gavage in water, or dermal application of neat or 50% aqueous solution. Oral and intravenous exposures were administered as a single dose and ranged from 10-1000 mg/kg bw. The highest dose was stated by authors to be double the developmental toxicity NOAEL for rats (500 mg/kg bw/day). Dermal exposures were conducted by applying ^{14}C-ethylene glycol to the shaved backs of animals and occluding for six hours, then rinsing with water. Undiluted ethylene glycol was applied at doses of 10 and 1,000 mg/kg bw/day in rats and 100 and 1000 mg/kg bw/day in mice. Authors attempted to use the same doses as the oral exposure experiments, but application of a neat 10 mg/kg bw dose to mice was not technically feasible. A 1000 mg/kg bw dose was also applied as a 50% aqueous solution to simulate automotive antifreeze and de-icing formulations. Concentrations of dosing solutions were verified. Blood, tissues, urine, feces and expired air samples were collected at intervals between 30 minutes through 96 hours post-dosing in 3-4 animals/group/time period and analyzed by gas chromatography (GC), high pressure liquid chromatography (HPLC), or liquid scintillation counting.

Toxicokinetic parameters following oral exposure are outlined in Table 2-3 for rats and mice. The authors concluded that plasma kinetics were linear (not dose-dependent) between the 10 and 1,000 mg/kg bw doses in both sexes of rat because mean residence time, area under the curve (AUC), clearance, terminal half life, and percent dose excreted as ethylene glycol were consistent. In contrast, female mice had inconsistencies between terminal half life, mean residence time, AUC, and clearance at these same doses which the authors suggest provided

evidence of non-linear (dose-dependent) plasma kinetics. **[The Panel noted that plasma kinetic data needs to be evaluated together with urinary excretion data. See utility statement below.]** Results with intravenous exposure were consistent with oral exposure results for each species. Following dermal exposure there was no evidence of dose-dependent changes in plasma kinetics or excretion patterns in either species or sex.

Table 2-3. Toxicokinetic Values Reported in Rats and Mice Exposed Orally to Ethylene Glycol by Frantz et al. *(30, 31, 32*).

Parameter	Values for Unmetabolized Ethylene Glycol Doses in mg/kg bw						
	10	**100**	**200**	**400**	**600**	**800**	**1000**
Female Rat:							
AUC (µg-h/g)	45.2	NA	NA	NA	NA	NA	4,012
$t_{1/2}^{\beta}$ (h)	2.5	NA	NA	NA	NA	NA	1.5
MRT_{∞} (h)	3.8	NA	NA	NA	NA	NA	2.5
$U_{\infty}^{\text{ethylene glycol}}$ (%)	0.2216	NA	NA	NA	NA	NA	0.2490
Cl_{oral} (ml/min/kg)	3.4	NA	NA	NA	NA	NA	3.9
Male Rat							
AUC (µg-h/g)	41.3	NA	NA	NA	NA	NA	6,041
$t_{1/2}^{\beta}$ (h)	1.4	NA	NA	NA	NA	NA	2.0
MRT_{∞} (h)	2.5	NA	NA	NA	NA	NA	3.6
$U_{\infty}^{\text{ethylene glycol}}$ (%)	0.2278	NA	NA	NA	NA	NA	0.2842
Cl_{total} (ml/min/kg)	4.0	NA	NA	NA	NA	NA	2.8
Female Mouse							
AUC (µg-h/g)	5.36	158.4	394.4	719.6/	NA	NA	2,501
$t_{1/2}^{\beta}$ (h)	0.3	0.5	0.5	0.5	NA	NA	1.1
$U_{\infty}^{\text{ethylene glycol}}$ (%)	0.1593	0.3321	0.2733	0.2883	NA	NA	0.3510
MRT_{∞} (h)	0.6	1.1	1.0	1.2	NA	NA	1.9
Cl_{oral} (ml/min/kg)	7.5	8.8	8.4	9.0	NA	NA	6.7

NA=Not analyzed; AUC=area under the concentration versus time curve; $t_{1/2}^{\beta}$=half life of elimination; MRT_{∞}=mean residence time; $U_{\infty}^{\text{ethylene glycol}}$ =percent dose excreted as ethylene glycol in urine; Cl_{oral}=clearance after oral dosing; Cl_{total}=total clearance

Excretion patterns observed in mice and rats by Franz et al. *(30, 31, 32*) are outlined in Table 2-4a and Table 2-4b. Following oral exposure the primary metabolites eliminated in rats and mice were CO_2 and glycolate. Cumulative ^{14}C excretion patterns over a 96-hour period changed with increasing dose which led study authors to suggest that oxidative metabolic pathways become saturated with high oral exposures. At the lowest oral dose (10 mg/kg bw), the primary and

secondary routes for elimination of ^{14}C were exhalation of $^{14}CO_2$ and urinary elimination of ^{14}C in urine, respectively (Table 2-4a). As dosages increased, urinary elimination of ^{14}C, exceeded exhalation of $^{14}CO_2$. Metabolic pathways in mice appeared to become saturated at lower doses when compared to rats. The shift to primarily urinary excretion occurred at doses exceeding 400 mg/kg bw, in female rats, at 1000 mg/kg bw in male rats, and at doses greater than 100 mg/kg bw in female mice. The shift in metabolism at higher doses of ethylene glycol resulted in the accumulation of glycolate. Ethylene glycol and glycolate were the main urinary metabolites detected and the ratio of glycolate to ethylene glycol increased proportional to dose. Percentages of ethylene glycol and metabolites in the urine of male rats are outlined in Table 2-4b. Oxalic acid was detected at low levels in the urine of male and female rats, but not mice. The absence of urinary oxalic acid in mice led authors to speculate that mice have a greater capacity than rats to metabolize low doses of ethylene glycol to CO_2.

Frantz et al. *(30, 31, 32)* noted that expired $^{14}CO_2$ and urinary ^{14}C were the primary and secondary metabolites eliminated over a 96-hour post-dosing period, respectively, in rats and mice dermally exposed to 10 or 1,000 mg/kg bw (neat or 50% solution) ethylene glycol. Because, there was no shift in excretion patterns with increasing dose (Table 2-4a), the authors suggested that metabolic pathways do not saturate at high dermal doses due to slow absorption through skin. The majority of radioactivity in urine following dermal exposure was associated with parent compound.

Table 2-4a. Excretion Patterns in Rats and Mice Administered ^{14}C-Ethylene Glycol by Oral or Dermal Route in Studies by Frantz et al. *(30, 32)*.

Exposure Route: Sex and Species	**Percent disposition of Exhaled CO_2/Urinary ^{14}C at each dose (mg/kg bw)**							
	10	**100**	**200**	**400**	**600**	**800**	**1000**	**1000 (50% solution)**
Oral:								
Female Rat	48/26	NA	NA	39/38	33/37	32/41	28/35	NA
Male Rat	42/26	NA	NA	39/20	34/26	30/26	27/42	NA
Female Mouse	55/24	42/43	31/44	26/45	NA	NA	22/56	NA
Dermal:								
Female Rat	13/8	NA	NA	NA	NA	NA	11/8	9/4
Male Rat	14/7	NA	NA	NA	NA	NA	14/8	6/5
Female Mouse	NE	10/7	NA	NA	NA	NA	16/12	10/5

NA=Not analyzed

Table 2-4b. Urinalysis Results for Ethylene Glycol and Metabolites in Male Rats.

Dose Group (mg/kg), Route	Percentage Interval Radioactivity Recovery					
	0-12 Hour Interval			12-24 Hour Interval		
	Oxalic acid	Glycolic acid	Ethylene Glycol	Oxalic acid	Glycolic acid	Ethylene Glycol
Oral:						
10	1.7	6.0	92.3	NP	NP	95.6
1000	NP	25.0	75.0	7.4	37.5	55.1
Dermal-Undiluted						
10	NP	100	NP	NP	12.8	87.2
1000	NP	100	NP	NP	2.8	97.2
Dermal: 50% dilution	NP	NP	NP	NP	NP	100 (at 24-36 hours)[a]
	NP	NP	NP	NP	NP	

NP=No peak detected
[a]First quantifiable interval

Strengths/Weakness:/Utility (Adequacy) for CERHR Evaluation Process: The Frantz et al. *(30, 31, 32*) studies provide useful toxicokinetic data over a relevant dose range. They provide data showing that ethylene glycol blood levels are nearly linear across a wide range of doses, but these data alone are deceptive because there are underlying non-linearities that are brought to light by the urinary excretion data. The excretion pattern indicates that the percent in urine jumps considerably from 10 to 100 mg/kg in mice, between 10 and 400 mg/kg in the female rat, and at higher doses in the male rat. The combination of ethylene glycol blood level data and the urinary excretion profile suggest that ethylene glycol oxidative metabolism is saturated but that the excess is excreted renally rather than accumulated in blood or tissues. The pattern also indicates increasing glycolic acid in urine as a percentage of dose over this dose range. Given that ethylene glycol oxidative metabolism to glycolic acid appears to become saturated, the most plausible mechanism for this excess of urinary glycolic acid is for removal of this metabolite to also become saturated leading to simultaneous increase in both ethylene glycol and glycolic acid in urine.

Thus, the Frantz data et al. *(30, 31, 32*) are important to show the saturation of both ethylene glycol and glycolic acid in rats and mice. It also tests whether bolus oral dosing is necessary for this phenomenon by employing high dermal doses in rats. In limited data (10 and 1000 mg/kg/d doses only) there does not appear to be any increase in ethylene glycol or glycolic acid in urine with the vast majority of urinary C^{14} remaining in the form of unmetabolized ethylene glycol. This suggests efficient removal of glycolic acid under dermal exposure conditions even at doses as high as 1000 mg/kg. From this we can conclude that the lower dose rate from dermal exposure does not present a great enough systemic ethylene glycol dose per unit time to saturate the oxidative enzyme systems.

Findings of the Frantz et al. *(30, 31, 32*) studies are consistent with results of an older study in which dose-related changes in excretion patterns were seen in male and female Fischer 344 rats administered ^{14}C-ethylene glycol (>99% purity) in saline intravenously at doses of 20, 200, 1,000, or 2,000 mg/kg bw *(41*).

Pottenger et al. *(33)* compared dose-related pharmacokinetics in adult pregnant (gd 10) versus nonpregnant female Sprague Dawley rats (n=4-5/group) administered a single gavage dose of ^{14}C-ethylene glycol (96.7% purity) in an aqueous solution. Doses in pregnant rats were 10, 150, 500, 1,000, or 2,500 mg/kg bw while non-pregnant rats were dosed with 10 or 2,500 mg/kg bw. Doses were at or below levels that produced developmental toxicity in rats. Pregnant rats were treated on gd 10 because it has been shown to be a sensitive period for ethylene glycol-induced developmental toxicity. Blood was collected prior to dosing and at 7 time intervals between 1 and 24 hours after dosing. Total urine eliminated was collected at 12 and 24 hours. Urine and blood samples were examined for ethylene glycol, glycolic acid, and oxalic acid by GC/MS. Table 2-5 lists the primary results for ethylene glycol and glycolic acid.

Table 2-5. Comparison of Ethylene Glycol and Glycolic Acid Toxicokinetics by Pottenger et al. *(33)*.

Parameter	Values for Ethylene Glycol/Glycolic Acid at Each Dose (mg/kg bw)				
	10	**150**	**500**	**1,000**	**2,500**
Pregnant					
T_{max} (h)	1/[a]	1/3	1/3	1/3	1/3
C_{max} (µg/g)	7.9/[a]	88.9/20.6	392/131	886/363	3,528/452
AUC (µg-h/g)	23/[a]	292/84	1,208/641	2,928/1,829	11,638/4,031
$t_{1/2}^{\beta}$ (h)	1.4/[a]	1.7/1.4	1.7/1	1.8/1.6	1.7/1.5
Total Urinary Elimination (% dose)[b]	14.95/0.88	27.86/1.18	41.92/12.43	39.64/20.13	37.64/32.79
Total ^{14}C-Urinary Elimination (% dose)[c]	15.83	29.71	54.97	60.33	71.09
Non-Pregnant					
T_{max} (h)	1/[a]	NA/NA	NA/NA	NA/NA	1/3
C_{max} (µg/g)	9.3/[a]	NA/NA	NA/NA	NA/NA	2,795/432
AUC (µg-h/g)	27/[a]	NA/NA	NA/NA	NA/NA	11,368/3,807
$t_{1/2}^{\beta}$	1.5/[a]	NA/NA	NA/NA	NA/NA	1.9/1.1
Total Urinary Elimination (% dose)[b]	14.62/1.36	NA/NA	NA/NA	NA/NA	37.63/31.36
Total ^{14}C-Urinary Elimination (% dose)[c]	16.35	NA	NA	NA	69.6

NA=Not analyzed
[a]Glycolic acid was below the quantifiable limit (2.1 µg/g) in blood at the lowest dose.
[b]Elimination of ethylene glycol/glycolic acid.
[c]Total elimination

Based on those results, the following conclusions were made by the authors:

- No significant differences in toxicokinetic parameters or urinary excretion profiles were observed between the pregnant (gd 10-11) and non-pregnant rats dosed with 10 or 2,500 mg/kg bw.
- A shift in blood glycolic acid toxicokinetics was noted at doses between 150-500 mg/kg bw as evident by C_{max} and AUC values that were not proportionate to increases in dose levels.
- Urinary excretion patterns were dose-dependent. Percentages of total urinary elimination increased with dose from about 16% at the 10 mg/kg bw dose to 70% at the 2,500 mg/kg bw

dose. The percentages of glycolic acid excreted in urine was disproportionate to dose starting at 500 mg/kg bw.

- Because shifts in urinary glycolic acid excretion paralleled changes observed in blood, dose-dependent changes in toxicokinetics and urinary excretion were most likely due to saturation of metabolic pathways and not saturation of renal elimination.
- Oxalic acid is not likely involved in the developmental toxicity associated with ethylene glycol since concentrations in blood were usually below the quantifiable limit of 4.9 μg/g blood. No dose-response relationship was noted the few times that oxalic acid was detected in blood. **[The Expert Panel did not feel the data were adequate to conclude about a possible role of oxalic acid in developmental toxicity, as noted in the strength/weakness/ utility statements below.]** Oxalic acid was excreted in urine at a constant fraction of administered dose (0.36-0.66%) and thus urinary levels increased with dose.

Strengths/Weakness: Significant physiological changes occur during pregnancy which could impact ethylene glycol disposition. The report by Pottenger et al. *(33*) was the first to address the issue of pregnancy and ethylene glycol disposition and as such, was important. Strengths of the study include a broad dose range that incorporated the NOEL for developmental toxicity in this species, a thorough pharmacokinetic analysis of both the parent compound and the two recognized major metabolites (glycolic acid and oxalic acid), and where expected, the fact that the data are in agreement with previous findings. There also are some notable weaknesses in the study. First, given the differences in the developmental expression of the ADH enzymes between the rat and human, combined with our uncertainty about the specific enzymes involved in human ethylene glycol metabolism, it is difficult to assess how well one might extrapolate these data to the human situation and the authors make no attempt to do so. Second, the study was limited to a narrow window of gestation, i.e., GD 10-11 in the Sprague Dawley rat. The rationale for choosing this gestational period was that previous studies had demonstrated this as a sensitive time-frame for ethylene glycol developmental toxicity. Another limitation of the study is that since exhaled breath was not collected, there is no attempt at mass balance. While it appears from the urinary data that there is a dose-dependent saturation of ethylene glycol and glycolic acid metabolism, the lack of exhaled CO_2 data and mass balance creates some uncertainty in this data set (e.g., changes in urinary metabolite levels may have been due to altered ethylene glycol bioavailability with increasing dose or due to measurement error). There also are concerns about the lack of concentration dependence for ethylene glycol and glycolic acid half-lives, which is incompatible with a zero-order process a being rate determining step. However, the ability of beta-elimination rate calculations to be sensitive to metabolic saturation is limited by the rapid elimination of ethylene glycol and glycolic acid by other pathways (renal elimination) and by the fact that key points in the glycolic acid blood decay curve were near the limit of detection. Finally, the reported blood oxalate data were at or near the limits of detection for the assay and were independent of dose. While the blood oxalate data are questionable, the urinary oxalate data show a linear increase in oxalate excretion over the entire dose profile suggesting no limitation on the production of oxalate. This may have been difficult to detect in blood due to rapid elimination into urine and/or due to calcium oxalate precipitation in blood.

Utility (Adequacy) for CERHR Evaluation Process: The utility of the Pottenger et al. *(33*) study for assessing human fetal exposure is uncertain. A search has not revealed a reliable assessment of whether ADH or other ethylene glycol metabolizing enzymes might change in the mother during pregnancy. Further, it is unclear whether a later point in gestation would have yielded substantially different toxicokinetic results due to the physiological changes that accompany later stages of pregnancy. If we assume no changes occur, then one might at least conclude that pregnancy would not have any profound impact on maternal ethylene glycol disposition. The maternal toxicokinetic handling of ethylene glycol and metabolites is a critical

factor in determining dose to the fetus, but we need to keep in mind the issue that ontological development of ADH in the fetus may be different across species, leading to the uncertainty that rat maternal toxicokinetic data may not extrapolate well when it comes to describing dosimetry to the human fetus. However, this study provides important confirmation of the saturation in glycolic acid oxidation in the rat that was suggested by Frantz et al. *(30, 31, 32*). In this case there is a well-defined dose range (ethylene glycol dose of 150-500 mg/kg) in which saturation takes place. While the Frantz et al. *(30, 31, 32*) data sets generally support the concept that percent urinary excretion increases with increasing ethylene glycol dose, the Frantz data show a less marked trend than that suggested by the Pottenger data. The combination of the Frantz and Pottenger data sets support the concept that developmental toxicity in rats (NOEL 500 mg/kg; LOEL 1000 mg/kg) appears to occur under conditions in which glycolic acid removal is saturated. At non-saturating doses (low oral doses, dermal exposure), the glycolic acid that is formed from ethylene glycol appears to be rapidly converted to further oxidation products and eliminated primarily as exhaled CO_2. At oral doses beginning somewhere between 150 and 500 mg/kg, glycolic acid oxidation appears to become saturated leading to the potential for its accumulation in blood. Given the evidence for increasing glycolic acid and oxalate exposure with dose throughout the dose range tested by Pottenger et al., this study cannot distinguish (nor was it designed to do so) which of these metabolites may be more important for developmental effects.

In a study conducted by Marshall and Cheng, *(37*) it was demonstrated that rat excretion patterns following inhalation exposure are similar to those following oral exposure. Two groups of 15 male and female Fischer 344 rats /sex (13-17-weeks-old) were exposed to ^{14}C-ethylene glycol (>99% purity) by nose only in the form of vapors (32 mg/m^3) for 30 minutes or aerosols (184 mg/m^3; MMAD=2.3 μm) on Ga_2O_3 particles for 17 minutes. **[The Expert Panel converted the inhalation doses to mg/kg bw/day values by using the minute volume of rats reported by study authors (1.3 mL/min/g bw). Exposure to vapor was estimated to be 1.25 mg/kg bw (32 mg/m^3 * 0.47 m^3 inhaled/24 hours * 0.5 hours * 1/0.25 kg, assuming 100% absorption). Exposure to aerosol was estimated at 7.1 mg/kg bw by using the same equation described above.]** Within the first four days following exposure, 63% and 20% of the ethylene glycol from the vapor exposure was eliminated as expired $^{14}CO_2$ and as urinary ^{14}C, respectively. For rats exposed to ethylene glycol aerosol, percentages of ethylene glycol eliminated by inhalation and urinary excretion were 70% and 11%, respectively. For vapor and aerosol exposure, 80% and 56% of urinary excretion, respectively, occurred during the first 24 hours and was comprised entirely of ethylene glycol. Metabolites found in urine at later time periods were not reported, although it appears that urine was analyzed for ethylene glycol metabolites. Information about absorption is included under Section 2.1.1.2.3.

Strengths/Weaknesses: The strength of this paper is that it utilized high dose inhalation exposure to probe ethylene glycol fate in a relevant animal model and it used sufficient numbers of animals (N=15 per dose group). Its main weakness is the limited identification of radiocarbon in urine, with no specific mention of assay for glycolic acid. Further, the radiocarbon data in blood and tissues may be confounded by the metabolism of ethylene glycol to formate and its subsequent utilization and labeling of tissues via the one carbon pool.

Utility (Adequacy) for CERHR Evaluation Process: The Marshall and Cheng *(37)* study provides perspective on inhalation exposures. The doses used in the study are far below the oral doses needed to demonstrate metabolic saturation and glycolic acid buildup which comports with the study's findings of relatively complete metabolism of ethylene glycol. The actual amount inhaled may be less since high concentrations of ethylene glycol may be irritating and result in a reflex decrease in respiratory rate. Given that Wills et al. *(26)* found that concentrations above 200 mg/m^3 were highly irritating and poorly tolerated by human volunteers, it would seem that the warning properties of ethylene glycol would prevent sufficiently high inhalation exposures that result in saturating conditions.

The effect of dose rate on ethylene glycol and glycolic acid blood levels in rats were studied by Carney et al. *(42)*. For this study, rats were dosed with ethylene glycol at 0, 1,000, or 2,000 mg/kg bw/day by either bolus sc injection or slow, continuous administration by an sc infusion pump. Rats receiving the bolus injections had higher mean blood levels of ethylene glycol (9.5 mM and 21.9 mM at each respective dose) and glycolic acid (3.3 mM and 6.3 mM at each respective dose) than rats receiving ethylene glycol by continuous infusions (ethylene glycol levels: 2.3 and 4.3 mM, respectively; glycolic acid levels: 0.1 and 1.0 mM, respectively). Complete details of this study and an evaluation of developmental toxicity through each exposure method are discussed in Section 3. **[The Expert Panel notes that since very little glycolic acid was found in plasma after the 1000 mg/kg/d pump infusion dose, it would appear that glycolic acid was readily metabolized and thus metabolism was not saturated. However, after 2000 mg/kg/d via infusion, there is evidence that glycolic acid did accumulate in plasma as the doubling of infusion dose led to a 10 fold increase in glycolate plasma levels. While this dose rate appears to have saturated glycolic acid removal, it yielded blood levels that were still below the glycolate levels seen after bolus dosing. However, this conclusion is based upon single time points after bolus (3 hours post exposure on gd 7, 9, 12, 15) and constant infusion (samples sometime on days gd 7, 9, 12, and 15) dosing. The major limitation of this design is that there is no estimate of AUC dose of ethylene glycol or glycolic acid. The AUC dose of ethylene glycol would have been beneficial for calculations that demonstrate the percent bioavailability of ethylene glycol from each dosing method. Without evaluating this, it is not completely clear that observed differences in toxicity that are attributed to dose rate aren't in part due to bioavailability differences between dosing methods. The AUC dose of glycolic acid is an important dose metric for testing how glycolic acid is related to developmental toxicity. The implication from this paper is that a peak concentration of glycolic acid over 2 mM is needed for developmental toxicity. With slow infusion of ethylene glycol, a glycolic acid peak or spike is not obtained, but the long-term cumulative (AUC) dose is the more relevant dose metric. The natural supposition from this paper is that the AUC doses for an equivalent ethylene glycol applied dose from the 2 dosing methods were roughly equivalent but that the peak dose was much greater from bolus dosing, which would explain the greater toxicity from bolus dosing. However, since AUC was not evaluated, this relationship is unclear and one cannot readily estimate the continuous dose needed to yield sufficient glycolic acid for toxicity. The paper does suggest that saturation of glycolic acid metabolism occurs somewhere between a constant infusion dose of 1000 and 2000 mg/kg bw/day. In contrast, Pottenger et al. *(33)* shows saturation from bolus dosing (gavage not sc in this case) occurs in the ethylene glycol dose range of 150–500 mg/kg bw. This would suggest that continuous dosing is approximately 3-10 fold less efficient at saturating glycolic acid metabolism as compared to bolus dosing.]**

Some limited studies in non-rodent species were briefly summarized in order to compare the findings with rodent studies.

Limited data in a series of studies conducted by McChesney et al. *(35*) demonstrated that monkeys produce the same metabolites as mice and rats. Two female rhesus monkeys (3.3 Kg) intravenously treated with 139 mg/kg ^{14}C-ethylene glycol in saline excreted ^{14}C primarily in urine and then expired air at 1 and 4 hours post-dosing; the temporal differences in sampling time do not permit direct comparisons with the cumulative values obtained in rodents over a period of days in the studies by Frantz et al. *(30, 31, 32*). In two separate experiments by McChesney et al., *(35*) the percentage of dose excreted in urine as ^{14}C and ethylene glycol at 0-48 hours post-dosing by gavage with ^{14}C ethylene glycol at 1 mL/kg bw **[1,109 mg/kg bw]**, ranged from 34.2 to 54.4% and 17.1 to 25.9%, respectively, in 4 female monkeys (5-7 kg). In one of the monkeys, it was determined that the percentage of dose excreted in urine as glycolic acid was 11.5% and as oxalic acid was 0.27% at 0-48 hours post dosing. Unidentified compounds represented 5.5% of the dose.

McChesney et al. *(35*) also describe a preliminary experiment conducted in 2 chimpanzees exposed to ^{14}C-ethylene glycol by IV at 2 ml/kg bw **[2,218 mg/kg bw]** or 1 ml/kg **[1,109 mg/kg bw].** At 9 hours post dosing, the animal exposed to the higher dose excreted 4% of the administered ^{14}C, 40% of this being in the form of ethylene glycol. The chimpanzee exposed to the lower dose excreted 28% of the administered ^{14}C in the first 8 hours, 30% in the form of ethylene glycol. In the 8-24 hour period it excreted an additional 11.3% of the administered ^{14}C, 17% as ethylene glycol. Expired $^{14}CO_2$ was not measured.

Strengths/Weakness/Utility (Adequacy) for CERHR Evaluation Process: These authors provide limited data on ethylene glycol disposition in rats and rhesus monkeys. The rat studies provide useful tissue distribution and mass balance information following an IV dose of 139 mg/kg of radiolabeled ethylene glycol. However, only one rat was followed long enough (24 hrs) to understand total metabolic disposition. In this one rat, only 14.4% of the dose was eliminated in expired air and 46.5% was excreted in urine. These values are considerably less for exhaled dose and greater for urinary elimination than one would expect at this low dose based upon Frantz et al. *(30, 31,(2*) who used oral dosing. It is possible that since IV administration does not involve first pass hepatic metabolism, that a greater percentage of administered dose is eliminated as unmetabolized ethylene glycol in urine rather than becoming completely metabolized and appearing in exhaled breath as CO_2. However, this is speculative since the data are from only a single rat.

The limited data in both rats and monkeys fall short of providing useful cross-species pharmacokinetic comparisons. Regarding monkeys, mass balance tissue and excretory data are provided only for 2 monkeys and only out through 4 hours post-dosing *(35*) (Table 2, Fig. 2). Plasma half-life data from two experiments in monkeys (n=3-4 per time point in each experiment) are useful in showing one compartment decay kinetics with a half-life range of 2.7-3.7 hrs, with the longer half-lives seen in monkeys that were 1 year older than monkeys in the first experiment which had the shorter half-life. The dose in these two experiments was 1 ml/kg (1109 mg/kg) by the oral route. The disposition of ethylene glycol in these animals is not well described, with no attempt at mass balance and only the amount of ethylene glycol in urine for 3 monkeys (16-20% of the dose) shown. Follow-up studies in 3 monkeys found that on average, 52% of the urinary elimination of a 1 mL/kg oral dose was in the form of ethylene glycol with the remainder being other metabolites. Urinary metabolism was further characterized in a single monkey dosed orally with 1 ml/kg. The urinary mass balance for this one monkey again indicated that 50% of radiolabeled in urine was parent compound, with approximately one-third appearing as glycolic acid (11.5% of total dose).

Overall, the variety of experiments reported by McChesney et al., *(35*) have the weaknesses of low numbers of animals on test, incomplete information reported for any given experiment, and no attempt at mass balance except in two experiments which looked primarily at short-term disposition in rats and monkeys. The most useful information is the reported half-life in monkeys (2.7-3.7 hours) which suggests somewhat slower clearance in monkeys than in rats given a similarly high dose of ethylene glycol [1.5-2 hrs; Frantz et al. *(30, 31, 32*)]. The evidence for glycolic acid excretion in a single monkey at the rate of 11.5% of the administered dose suggests that monkeys may eliminate less ethylene glycol as glycolic acid than seen in rats (e.g., Pottenger et al. *(33*) showed 20% eliminated as glycolic acid after a similar oral dose). However, the monkey data are too limited (only 1 animal, 1 dose, no mass balance) to provide useful quantitative information regarding glycolic acid excretion or to make cross-species comparisons. The utility of this excretion data is simply that it provides evidence for substantiative glycolic acid production and elimination in monkeys.

Regarding the McChesney et al.'s chimpanzee data, the high dose animal died after 9 hours and so only very limited toxicokinetics data are available from this animal (no blood data, no mass balance, only 4% of the dose accounted for in urine). Urine was collected from the other chimpanzee out to 24 hrs post dosing, at which point only 39% of the dose was accounted for in urine, leading to the possibility of extensive metabolism and ethylene glycol elimination as CO_2 for the majority of the dose. This would be an interesting finding given that at this high dose (1109 mg/kg bw) one would expect less exhalation (and thus less complete metabolism) and more urinary elimination based upon the rat studies *(33*). However, there is data from only one chimpanzee tested at only one dose, and there was no collection of exhaled radiolabel for this animal. Thus, these data cannot be relied upon.

Some limited information on the metabolism of ethylene glycol in rabbits and a comparison to other species is available from a study by Gessner et al. *(43*). In that study, ^{14}C-ethylene glycol (98±2% purity) in water was administered orally or by subcutaneous injection to chinchilla rabbits, albino rats, guinea pigs and cats **[sex not specified]**. In rabbits (n=1/dose/time period) administered oral doses of 124-2,000 mg/kg bw, approximately 20-30% of the radioactivity was excreted in the urine within 2 days. At the lowest dose (124 mg/kg bw) a large proportion of the ^{14}C was eliminated in the expired air as carbon dioxide, 42% within one day and 60% within 3 days. **[The percentage of ^{14}C eliminated in expired air was not reported for the remaining doses; it was not clear if the values were not measured or were below limits of detection.]** Ethylene glycol was found to be the major urinary metabolite at 24 hours following oral administration of ethylene glycol at 25 mg/kg bw in two rabbits and 125 mg/kg bw in 1 rabbit. Ethylene glycol represented 6-15% and 10% of the 25 mg/kg bw and 125 mg/kg bw doses, respectively. Trace levels of oxalic acid (0.01-0.11% of dose) were also present. No other ethylene glycol metabolites were detected including glycolic acid. Excretion of oxalate in urine does appear to vary between cats, rats, rabbits and guinea pigs. Following administration of ethylene glycol by an unspecified route at doses of 100-1,000 mg/kg bw/day, cats were found to have the highest levels of urinary oxalate (0.7-3.7% of dose); rabbits and guinea pigs had the lowest levels of oxalate in urine (~0.05% of dose) and rats had intermediate levels (0.5-1.1% of dose).

Strengths/Weakness: This study is limited by the small numbers of animals on test at any dose level, the lack of mass balance information (including no CO_2 measurements for most dose groups), and the potential method detection limit issues which may have prevented the finding of glycolic acid and other metabolites in urine. However, the study does offer the suggestion of metabolic saturation of ethylene glycol in several species as evidenced by a trend towards increasing urinary excretion with increasing dose.

Utility (Adequacy) for CERHR Evaluation Process: The toxicokinetics evaluations in rats and rabbits were useful in that a broad range of doses were evaluated, but only one animal was on test at any given dose level. The results suggest an increasing amount of radiolabel in urine and less in expired air with increasing dose which is expected from other studies as well. Analysis of radiolabel in urine suggested that ethylene glycol constituted the majority of urinary excretion in two rabbits dosed with 25 mg/kg and 45% of urinary excretion in one rabbit dosed with 125 mg/kg. While trace levels of oxalic acid were detected in all three rabbits, other urinary metabolites (e.g., glycolic acid) were not detectable. This may be related to high detection limits for the analytical method for these metabolites; the minimum detection limits were not stated but appear to be based upon melting point determinations following a series of metabolite derivatizations; the sensitivity and percent recovery of these methods was not reported and is uncertain.

Hewlett et al. *(36*) studied excretion patterns in rats and dogs administered ethylene glycol in water by gavage. Twenty-four male Sprague-Dawley rats (3/time point) were dosed with 2000 mg/kg bw and one male and one female mixed-breed dog were dosed with 1,000 and 1,036 mg/kg bw, respectively. Ethylene Glycol and glycolate levels in blood and urine were measured at various time intervals between 1 and 24 hours by GC or HPLC. Glycolate levels peaked between 4-6 hours post-dosing in both species. Renal excretion of ethylene glycol in rats was almost complete in the first 6 hours, while glycolate excretion required another 6 hours. In rats and dogs respectively, about 20% and 30% of the dose was excreted as ethylene glycol and 5% and 4% as glycolate within 24-30 hours. The study authors concluded that toxicokinetics were similar in both species. **[According to the Expert Panel, renal clearance of ethylene glycol needs to be considered an important pathway of ethylene glycol elimination, as indicated by these results and by a recent case of human ethylene glycol poisoning without renal failure] *(44*).**

Strengths/Weakness/Utility (Adequacy) for CERHR Evaluation Process: While the study authors concluded that ethylene glycol kinetics is similar between rats and dogs, Figure 1 of the Hewlett et al. *(36*) study shows essentially the same peak ethylene glycol plasma level in both species in spite of the fact that rats received approximately 2 times the dose given to dogs. This suggests a significant difference between the species. The shorter half-life in rats provides at least a partial possible explanation for the similarity in plasma ethylene glycol levels across species (i.e., more rapid elimination in rats would tend to curtail peak blood levels relative to dogs). The use of only a single dose per species, the lack of measurement of exhaled breath or mass balance limit the utility of the rat data for risk assessment. Added to these limitations, the use of only 2 dogs limits the conclusions that can be made concerning ethylene glycol kinetics in this species. However, an important data set contained within this paper is the evidence of ethylene glycol-induced diuresis in rats immediately after the 2000 mg/kg dose followed by a rapid decline in urine output to below control levels. This suggests impaired renal function by 8-12 hours post-dosing. The implications of this impairment in renal function at this relatively late point in the time course of ethylene glycol disposition is not clear, but it may compound the risks from high dose ethylene glycol exposure.

Blood glycolate levels in ethylene glycol treated animals were measured in a limited number of studies and are presented below in Table 2-6.

Table 2-6 demonstrates that high bolus doses of ethylene glycol produce relatively high glycolic acid levels in blood (5-13 mM). One study *(33*) suggest that from a dose-response perspective, there is a super-linear increase with dose in rats. Consistent with the blood trend data was a

marked increase in urinary glycolic acid excretion at doses above 150 mg/kg bw Levels of blood glycolic acid observed in human poisonings are outlined in Section 2.1.3.1.

Table 2-6. Maximum Levels of Glycolic Acid in Blood Following Gavage Exposure to Ethylene Glycol.

Sex and Species (Exposure Route)	Ethylene Glycol Dose (mg/kg bw)[a]	Blood Glycolic Acid Level (mM)	Reference
Female Sprague Dawley rats (gavage).	2,500	5.7	Pottenger et al. *(33)*
Pregnant Sprague Dawley rats (gavage).	150 500 1,000 2,500	0.27 1.7 4.8 5.9	Pottenger et al. *(33)*
Pregnant Sprague Dawley rats (gavage).	2,500	8.8	Carney et al. *(45)*
Pregnant Sprague Dawley rats (bolus sc injection)	1,000 2,000	3.3[b] 6.3[b]	Carney et al. *(42)*
Pregnant Sprague Dawley rats (slow, continuous sc infusion)	1,000 2000	0.1[b] 1.0[b]	Carney et al. *(42)*
Male Sprague Dawley rats (gavage).	2,000	13	Hewlett et al. *(36)*
Male and female mixed-breed dogs (gavage).	1,000-1,036	8	Hewlett et al. *(36)*

[a][Doses in Pottenger et al. *(33)* study converted from original units using a glycolic acid molecular weight of 76.05 and by assuming that the specific gravity of blood is ≅1. Example calculation for 2500 mg/kg bw exposure in pregnant rats: 452 μ glycolic acid/g blood x 1 g blood/1 mL x 1000 mL/1L x 1 mg/1000 μg x 1 mmol glycolic acid/76.05 g = 5.9 mM].

[b]These were not necessarily peak glycolic acid levels

Two studies provide evidence of a possible second metabolic pathway for ethylene glycol in the rat. Kukielka and Cederbaum *(46)* found evidence that in the rat "…ethylene glycol is oxidized to formaldehyde by an oxidant derived from H_2O_2 and nonheme iron, and that cytochrome P-450 may function to generate the H_2O_2 and to catalyze reduction of the nonheme iron." Based on increased levels of radical adducts in urine and bile of rats treated with ethylene glycol and 4-methyl pyrazole, Kadiiska and Mason *(47)* suggested there may be a second minor pathway for ethylene glycol that may compete with alcohol dehydrogenase.

2.1.3.3 Developmental- and Species-Specific Variations in Metabolism and Enzyme Activities

Because metabolites of ethylene glycol are responsible for the systemic toxicity observed with high exposures, varying activities of metabolic enzymes such as ADH and ALDH may affect toxicity. A number of studies examined ADH or ALDH activity in human placenta, age related activity of the enzymes, and species specific differences in humans and rats. Though the focus of most studies was ethanol, the studies are relevant to ethylene glycol since both chemicals are substrates of ADH and ALDH. Therefore, a brief review of the data was conducted by the Expert Panel.

2.1.3.3.1 Placental Metabolic Capacity

Studies in humans and rodents suggest that the placenta has extremely limited capacity to metabolize ethylene glycol and its metabolites. Pares et al. *(48)* isolated Class III ADH from full term human placenta and found it had low activity for ethanol and a K_m value for octanol that was 100 times higher compared to the Class I ADH enzyme found in human liver. Zorzano and Herrera *(49)* found that ALDH from full term human placentas had a lower activity and V_{max} and a higher K_m value than ALDH isoenzymes from liver.

In rats, placenta was found to have no ADH activity and ALDH activity in placenta was found to be 4-7% of liver ALDH activity *(50)*. The findings of the study were consistent with several older studies that are referenced in the introduction and discussion.

2.1.3.3.2 Developmental Aspects of Metabolic Capacity

Activity of ADH and ALDH was found to vary with developmental stage. Pikkarainen and Raiha *(51)* measured *in vitro* alcohol dehydrogenase (ADH) activity in the livers of human fetuses, children, and adults (n=1–3/age group) using ethanol as a substrate. The ADH activity in 2-month-old fetal livers was about 3–4% that of adults. In 4–5 month old fetuses, ADH activity was roughly 10% that of adults, and in infancy, activity was about 20% that of adults. ADH activity increased in children with age, and at 5 years of age reached a level that was within the ranges noted for adults. Great variation was noted in adult ADH activity. Somewhat different results were reported subsequently by Smith et al. *(52)* who examined liver ADH activity using ethanol as a substrate and also examined the ontogeny of individual ADH class I isoforms. They reported total ADH activity in 9-22 week fetal liver that was 30% of adult values and in premature infants and children less than 1 year of age, activity was 50% of adult values. Individual enzyme activity was determined using starch gel electrophoresis with an *in situ* assay. A total of 222 liver samples were assayed, 56 from fetuses (9-22 weeks gestation), 37 from premature infants and infants less than 1 year of age, and 129 from adults greater than 20 years of age. In fetal liver samples with a mean gestational age of 11 weeks, only the ADH1A enzyme was detectable. By 17 weeks, both ADH1A and ADH1B were measurable, although ADH1A predominated. By 19 weeks, products from all three loci were observed, with ADH1A greater than ADH1B, and ADH1B greater than ADH1C. At 30 weeks, ADH1A and ADH1B levels were equivalent, but still greater than ADH1C, but by 36 weeks, ADH1B expression dominated. In the adult, hepatic ADH1A expression was nondetectable, whereas expression from the *ADH1B* and *ADH1C* loci were equivalent. Interestingly, this progressive change in expression was tissue-specific. In lung, there were no observed differences between the fetal and adult samples and only ADH1C was detectable. ADH expression in the intestine and kidney was low and did not change appreciably with age.

Sjoblom et al. *(50)* found that in Wistar rats ADH activity in liver was not detected at birth, was 3% of adult activity on pnd 20, and continued to increase with age to 65% and 82% of adult activity on pnd 21 and 47, respectively. Similar developmental patterns were noted for ALDH in rat liver.

[According to the Expert Panel, it would appear that human liver ADH is expressed much earlier in development and may well contribute to ethylene glycol metabolic disposition. However, given the paucity of knowledge regarding isoform specificity towards ethylene glycol, it is uncertain how these data on ethanol metabolism might be extrapolated. If one assumes that the enzyme most active in ethanol metabolism, ADH1B, also is most active in ethylene glycol metabolism, then one would not predict significant fetal metabolism until later in gestational development (20-36 weeks).]

2.1.3.3.3 Hepatic Metabolic Capacity in Humans Versus Rats

Zorzano and Herrera *(53, 54*) found different ADH isoenzymes in liver homogenates from humans (class I ADH) and rats (ADH-3) which differed greatly in kinetic properties. Using ethanol as a substrate at a pH of 10.5, activity, K_m, and V_{max} in humans was measured at 6.24 Units/g tissue, 2.10 mM, and 7.70 Units/g tissue, respectively, while activity, K_m, and V_{max} in rats was measured at 2.72 Units/g tissue, 1.02 mM, and 2.96 Units/g tissue, respectively. Two different low K_m ALDH isoenzymes were found in humans and rats but they had similar activities using acetaldehyde as the substrate at pH 8.8 (humans: K_m=9 μM and V_{max}=0.85 Units/ g tissue; rats: K_m=10 μM and V_{max}=0.87 Units/ g tissue).

2.1.3.3.4 Genetic Polymorphisms

Reviews by Agarwal, *(55*) Bosron and Li, *(56*) Pietruszko *(57*) and Burnell et al. *(58*) discussed genetic polymorphisms for ADH and ALDH in humans. Class I ADH, the primary ADH in human liver, is a dimer composed of randomly associated polypeptide units encoded by 3 loci (*ADH1A, ADH1B*, and *ADH1C*). Polymorphisms resulting in altered phenotypes are observed at the *ADH1B* (*ADH1B*2* and *ADH1B*3*) and *ADH1C* (*ADH1C*2*) loci. The *ADH1B*2* allele is estimated to occur in15% of Caucasians of European descent, 85% of Asians, but less than 5% of African Americans. Fifteen percent of African Americans have the *ADH1B*3* allele, while this variant is essentially absent in other ethnic groups. Both the ADH1B*2 and ADH1B*3 enzymes have V_{max}, values for ethanol that are 100-fold higher than that exhibited by ADH1B*1. The ADH1B*2 and ADH1B*3 differ in that their affinities for ethanol are approximately 20- and 70-fold lower than ADH1B*1, respectively

There are two primary ALDH isoenzymes in human liver, ALDH2 (also referred to as E_2 , ALDHI, or $ALDH_2$) and ALDH1 (also referred to as and E_1 , ALDHII, or $ALDH_1$) *(55-57*). About 50% of Japanese and Chinese carry a phenotypically null variant of the ALDH2 enzyme.

[The Expert Panel concluded that given the roles for ADH, ALDH, and potentially, CYP2E1, in the disposition of ethylene glycol, the issues of species differences, developmental differences, and genetic variability is of significant relevance to conducting risk assessment and/or to the development of PBPK models.]

2.1.4 Elimination

2.1.4.1 Humans

The half-life for elimination of ethylene glycol in humans is reported as 2.5-8.4 hours, and after 24-48 hours, little to no ethylene glycol can be detected in urine or tissues *(5*). Evaluation of human ethylene glycol elimination data are typically complicated by treatment with ethanol to prevent further metabolism of Ethylene glycol to glycolic acid *(39, 44, 40*). This prevents a direct comparison of ethylene glycol half-life in rats and humans.

Cheng et al. *(44*) measured renal clearance in a 25-year-old man who drank about 280 mL of antifreeze containing 95% ethylene glycol and was treated with ethanol and hemodialysis. It was found that the normally, functioning kidney greatly contributes to the excretion of ethylene glycol. Mean renal clearance was measured at 27.5 mL/minute, fractional excretion was about 20%, and the half-life for renal clearance of ethylene glycol was estimated at 18 hours. The findings were in contrast to renal clearance rates of 1-4 mL/minute reported for patients with acute renal failure. In another case study, post-ethanol treatment serum toxicokinetics suggested a half-life of approximately 15 hours, *(40*) a value that is consistent with the 18 hour half-life for renal elimination reported by Cheng et al. *(44*). In human cases before dialysis was employed,

glycolic acid clearance was generally very slow *(39*). These slow clearances may be due to a combination of high blood glycolate levels which saturated metabolism, and impaired renal function. In one case involving overdose in a 2 year old girl, glycolate levels were high but the decline from serum (without dialysis) suggest a half-life of approximately 10 hours *(40*).

2.1.4.2 Animals

Ethylene glycol is rapidly cleared from blood with elimination half-lives reported at 1.1 hours in mice *(32*), 1.5-2.5 hours in rats *(30, 32, 33*) and about 3 hours in monkeys *(35*) and dogs *(36*). The half-life of both ethylene glycol and glycolic acid are dose-independent *(30, 31, 32, 33*), such that even high glycolic acid concentrations in blood that were likely in excess of the Km for glycolic acid metabolisms were rapidly cleared. Rapid clearance under saturating conditions with limited metabolic removal suggests that renal elimination can effectively remove both parent compound and metabolite. This however, may not be the case in subjects with renal impairment, which could result from calcium oxalate formation; this could compound the reproductive/ developmental toxicity from ethylene glycol. As discussed in greater detail in Section 2.1.3, ethylene glycol is primarily eliminated in urine as parent compound or glycolic acid and in exhaled air as CO_2; the percentage of each elimination product is highly dependent on dose rate and route of exposure *(30, 31, 32, 33*).

2.2 General Toxicity

2.2.1 Human Data

2.2.1.1 Acute Exposure

Acute ethylene glycol toxicity in humans is well characterized and numerous case studies have addressed the topic. Such case studies contribute little to the understanding of developmental and reproductive effects. Therefore this section is derived primarily from reviews conducted by LaKind et al. *(59*), ATSDR *(5*), NTP *(23*), and Carney *(24*). Numerous human deaths resulting from intentional or accidental ingestion of ethylene glycol have been documented. The lethal oral dose for humans has been estimated at 1400-1600 mg/kg bw. However, the estimation of acute lethal doses in humans is uncertain, because the exact quantity ingested cannot be quantified. Toxicity associated with acute oral exposure to ethylene glycol is characterized by at least three distinct stages that can overlap. Death can occur during any of the stages. Stage I occurs within 30 minutes to 12 hours following intake and primary symptoms include central nervous system (CNS) depression and gastrointestinal upset. Individuals in Stage I appear to be drunk and depending on the dose, CNS symptoms can include ataxia, slurred speech, somnolence, and convulsions. Metabolic acidosis is said to occur during Stage I or Stage II. Stage II occurs at 12-72 hours following ingestion and is characterized by cardiopulmonary toxicity. Observed at this stage is severe metabolic acidosis characterized by reductions in blood pH and bicarbonate levels. Severe serum hyperosmolality and increased anionic gap can also occur. Cardiopulmonary symptoms during this stage may include tachypnea, hypernea, tachycardia, cyanosis, pulmonary edema, or cardiac failure. Metabolic acidosis is thought to be the cause of these symptoms. Another possible cause of symptoms is hypocalcemia that can occur as oxalate binds with calcium. Stage III, that occurs at 24-72 hours following ingestion, is characterized by renal toxicity. Calcium oxalate crystal deposition within kidneys is thought to be the major contributing factor to renal failure. Additional symptoms that can occur during Stage III include flank pain and polyuria later followed by oliguria. Histological examination of kidneys reveals both tubular necrosis and oxylate crystals. Neurological symptoms that uncommonly occur six or more days after ethylene glycol ingestion suggest that there may be a fourth stage of toxicity

involving cranial nerves. The symptoms include deafness, facial paralysis, and other neurologic sequelae. Autopsy material from a person in this newly discovered fourth stage revealed dense refractile crystal deposition along portions of the seventh and eighth cranial nerves *(60*).

Data on acute ethylene glycol toxicity resulting from inhalation or dermal exposure is very limited.

In a controlled study, nasal and throat irritation were noted following short-term inhalation exposure to ethylene glycol at concentrations exceeding 140 mg/m^3. Ethylene glycol levels above 200 mg/m^3 were intolerable *(26*).

ATSDR *(5*) stated that acute dermal exposure to ethylene glycol is most likely to occur through products such as antifreeze but is not likely to lead to toxic effects. LaKind et al. *(59*) reported that ethylene glycol appears to be a mild skin irritant but not a skin sensitizer.

2.2.1.2 Repeated Exposures

The data on repeat exposure to ethylene glycol toxicity in humans is limited and not as extensively covered in the literature as acute toxicity data. Therefore, original studies were reviewed to evaluate human toxicity in controlled *(26*) and occupational studies *(19, 20*). This section was not broken down according to exposure routes since exposures likely occurred through multiple routes.

In a controlled study by Wills et al., *(26*) 19 male prisoners were continuously exposed to aerosolized ethylene glycol for 20-22 hours/day for 30 days. The study was conducted in a prison ward hospital that was converted to an exposure chamber. Ethylene glycol mists were generated using 3 air conditioning units. Air concentrations were measured at 5 locations within the chamber by analyzing ethylene glycol levels in air collected from evacuated polyethylene bottles. Mean daily concentrations of ethylene glycol were 3-67 mg/m^3 and mean weekly concentrations were 17-49 mg/m^3. The diameter of mist droplets was 1-5 microns. The control group consisted of 14 male prisoners. Ten of those men were never exposed to ethylene glycol, while 4 were just exposed to ethylene glycol for 7 days in a pilot study. Neurobehavioral effects were measured and electrocardiographs and electroencephalographs were conducted prior to exposure and following 2 and 4 weeks of exposure. Blood samples were collected before exposure and approximately every 2-3 days during exposure. Urine samples were collected daily to check for oxalate crystals, and twice weakly for urinalysis. **[Statistical analysis was not described.]** Exposure resulted in individual blood serum levels of 0.094-0.18 mg/ml ethylene glycol and urinary levels of 0.021-0.077 mg/ml ethylene glycol. Ethylene glycol levels were similar in unexposed controls (0.09-0.21 mg/ml in serum and 0.017-0.077 mg/ml in urine). Exposure resulted in no significant changes in urinalysis, hematological, or blood chemistry (including urea nitrogen, creatinine, and plasma pH) parameters, or in neurobehavioral, heart, or brain function. Subjects did occasionally complain of headaches and lower back pain.

Gerin et al. *(19*) conducted a study to measure ethylene glycol exposure and kidney function in 33 male Canadian aviation workers (21-52-years-old) exposed to ethylene glycol-based de-icing fluid. Details about the exposure portion of the study are included in Section 1. The study was conducted in Quebec from January to March in 1992. Personal exposures to ethylene glycol vapors and mists were measured at <2.5-22 mg/m^3 and <17 -190 mg/m^3, respectively. Post-shift levels of ethylene glycol in urine ranged from <5-129 mmol/mol creatinine. Diethylene glycol was sometimes detected in air or urine samples at levels that were about one-tenth the ethylene glycol concentrations. Some of the workers wore paper masks that offered some protection against mists but not vapors. Possible confounding factors considered included demographics,

work activities, health problems, analgesic intake, smoking habits, alcohol intake, and non-occupational exposures to solvents and ethylene glycol. T-tests were conducted to analyze time-related (temporal) data, such as urine levels before and after the shift. Subgroup exposure (dose-response) data were analyzed by analysis of variance (ANOVA), and Fisher's exact test. Kidney function was assessed by measuring pre- and post-shift urinary levels of β-N-acetyl-glucosaminidase, albumin, β-2-microglobulin, and retinol-binding protein. There were some significant associations between kidney function parameters and ethylene glycol exposures. However, no consistent effects were observed and most values were within normal limits. The authors concluded that there was no evidence of acute or chronic renal toxicity related to ethylene glycol exposure in this study. They also noted that the statistical power of the study may have been limited due to small sample size and wide variations in the renal function parameters examined.

Laitinen et al. *(20)* examined exposure to ethylene and propylene glycol and possible indicators of biochemical renal effects in Finish motor servicing workers. Details about the exposure part of the study are discussed in Section 1. Ten male mechanics from 5 different garages participated in the study. The only skin protection used by some workers was leather gloves. Ten age matched office workers served as controls. **[Ages of subjects and possible confounding factors were not discussed.]** Urine samples were collected after the work shift and analyses results were compared to controls. Differences between groups were evaluated by Student's t-test. As discussed in Section 1, urinary ethylene glycol levels were significantly higher in mechanics (7.3 vs. 1.7 mmol/mol creatinine, respectively). Urinary oxalic acid levels were slightly higher in mechanics (47 vs. 36 mmol/mol creatinine in controls) but differences between controls did not reach statistical significance. Of the biochemical parameters examined in urine, glycosaminoglycans levels were significantly lower in controls. Urinary calcium concentration and succinate dehydrogenase activity were marginally reduced in mechanics, but the effects were not statistically significant. Urinary levels of ammonia were higher in exposed workers **[The effect was said to be significant in the text but not the table.]** According to the study authors, increased ammonia excretion is typically observed with chronic acidosis.

LaKind et al. *(59)* noted a case study that reported nystagmus and decreased blood cell counts in 38 women exposed to unknown concentrations of ethylene glycol vapors from a heated ethylene glycol-containing mixture. It was noted by LaKind et al. *(59)* that exposures were not known and other occupational conditions were not considered.

2.2.2 Experimental Animal Data

2.2.2.1 Oral Exposure

The majority of acute toxicity studies provide no information about ethylene glycol effects on reproductive organs. For that reason, much of the information in this section was obtained from reviews by LaKind et al. *(59)*, ATSDR *(5)*, and Carney *(24)*. Based on reported minimal lethal doses (MLD), it has long been assumed that most laboratory animals are less sensitive than humans to acute ethylene glycol exposure. For example, the minimum lethal oral dose in rats, 3800 mg/kg bw is much higher than the minimum lethal dose in humans, 1400-1600 mg/kg bw/day. However, the assumption of increased human sensitivity has recently been questioned due to an inability to accurately determine doses received by humans in accidental or intentional poisoning exposures *(61)*. Table 2-7 outlines LC50's and minimum lethal doses reported in various animal species.

Table 2-7. Comparison of Oral Minimum Lethal Doses (MLD) and LD50's in Animals.

Species	Oral MLD(mg/kg bw/day)	Oral LD50 (mg/kg bw)
Rat	3800[b]	4000-8790[a]
Mouse	Unknown	8400-15,400[a]
Guinea Pig	Unknown	6610-8200[a]
Rabbit	Unknown	5000[b]
Dog	6700-7400[c]	>8000[b]

[a]LaKind et al. *(59)*
[b]Carney *(24)*
[C]NTP *(23)*

Toxic effects induced by ethylene glycol exposure in animals are similar to those observed in humans. Like humans, toxicity in animals proceeds in three stages that include: 1) central nervous system effects, 2) metabolic acidosis and cardiopulmonary effects, and 3) renal toxicity. Repeated exposures in animals also result in the same types of symptoms. Table 2-8 lists some common symptoms of ethylene glycol toxicity in humans and the animals species for which that symptom has also been observed either under acute or repeated exposure conditions.

Table 2-8. Symptoms of Acute Ethylene Glycol toxicity Noted in Various Animal Species.

Symptom	Species That Symptom was Observed In
Central Nervous System Toxicity	Rat, Mouse, Guinea Pig, Rabbit, Dog
Metabolic Acidosis	Dog, Primate, Rat
Pulmonary Toxicity	Dog, Mouse, Rat, Guinea Pig
Cardiac Toxicity	Dog
Calcium Oxylate Crystals Formation	Cat, Dog, Rat, Monkey, Rabbit, Pig
Renal Lesions	Dog, Rat, Mouse, Guinea Pig
Renal Failure	Dog, Cat, Rabbit

Numerous repeat dose studies have examined toxicity of ethylene glycol in various species. The studies consistently demonstrate the kidney as the cardinal target organ. The liver has also been noted as a target organ in some studies *(23)*. The Expert Panel did not conduct an exhaustive review of repeat dose toxicity studies since the majority of the studies did not include a histological examination of reproductive organs. However, an NTP *(23)* report pointed out some interesting observations about species and sex specific aspects of ethylene glycol exposure that warrant discussion.

Male rats were found to be more susceptible than female rats to ethylene glycol-induced toxicity. Subchronic *(62, 63, 64)* and chronic *(65, 66)* dietary studies in male and female Fischer 344 rats have consistently demonstrated a higher mortality rate, greater severity of kidney lesions and oxalate crystal deposition, and higher levels of blood urea nitrogen and creatinine in males. For example, in subchronic studies, kidney lesions were noted in males fed a diet with 25,000 ppm and females fed a diet with 50,000 ppm ethylene glycol. Health Canada *(9)* and ACGIH *(67)* noted studies that demonstrated reduced kidney stone formation and urinary oxalic acid in castrated versus intact male rats administered 0.5% ethylene glycol in drinking water for 28 days; administration of testosterone to castrated rats resulted in a reversal of effects.

Sensitivity was found to vary widely among different species. Two chronic studies conducted in B6C3F1 or CD-1 mice found no or less severe lesions in mice compared to male rats *(23, 65*). All of these studies are reviewed in detail below. Variability in species toxicity was also noted in developmental toxicity studies conducted in mice, rats and rabbits. Tyl et al. *(68*) noted that more than 40% of rabbits died following gavage treatment with 2000 mg/kg bw/day, while no rats or mice died following gavage dosing with 5000 and 3000 mg/kg bw/day, respectively *(69*). In a developmental toxicity screening study, there was a 10% death rate in mice gavage treated with 11,090 mg/kg/day of ethylene glycol *(70*). These data indicate that rabbits respond somewhat differently to ethylene glycol than rats and mice. A paucity of information on the physiologic and metabolic response to ethylene glycol in rabbits allows one only to speculate about potential mechanisms. Renal oxalate deposition certainly is a striking finding in rabbits treated with high levels of ethylene glycol and is a plausible mechanism of toxicity. However, a cause and effect relationship for oxalate deposition and toxicity has yet to be established *(39*). Also, it is not known if rabbits develop severe metabolic acidosis as most other species do *(36*).

CERHR reviewed repeat dose oral exposure studies conducted in rodents by Melnick *(62*) NTP *(23*), and Gaunt et al. *(63*) and in monkeys by Blood et al. *(71*) since they included histological examinations of reproductive organs. A subchronic rat study conducted by Robinson et al. *(64*) was also reviewed since it is one of the key studies examining kidney effects. Some chronic studies conducted to evaluate carcinogenicity *(23, 65*) are reviewed in Section 2.4. These rodent subchronic and chronic studies represent the key systemic toxicity studies that were presented in reviews by ATSDR *(5*) and Health Canada *(9*).

Melnick *(62*) and NTP *(23*) reported the results of a 13-week study in male and female B6C3F1 mice (age 63 days) exposed to ethylene glycol (>99% pure) through their diet. The purpose of the study, conducted according to Good Laboratory Practices, was to determine doses to be used in a 2-year study described under Section 2.4. Ten animals/sex/group, were exposed to 0, 3,200, 6,300, 12,500, 25,000, or 50,000 ppm ethylene glycol in food. **[CERHR estimated doses based on actual mean body weights of animals at start and end of experiment (23-31 g for males and 18-25 g for females) and food intake rate (~8 g/day) measured during weeks 1-13 of the 2-year NTP *(23*) study. The CERHR dose estimates were 830-1110, 1630-2190, 3230-4350, 6450-8700, and 12,900-17,400 mg/kg bw/day in males and 1020-1420, 2020-2800, 4000-5560, 8000-11,110, and 16,000-22,220 in females from the low to high dose groups, respectively.]** Doses were selected based on effects reported in the literature. Observed endpoints included survival, body and organ weight, clinical signs, necropsy, hematology, blood chemistry, urinalysis, and histopathology. Incidence data were evaluated by logistic regression, the Fisher exact test, the Cochran-Armitage trend test, pairwise comparisons, and determining overall dose response trends. Continuous data were analyzed by the Williams', Dunnett's, or Jonkheere's test. There were no differences in final body weight, organ weights, or clinical findings. No lesions in reproductive tissues (ovary, uterus, prostate testis; preserved in 10% formalin) were reported following examination of primarily control and high dose animals. Mild treatment related lesions were observed in the liver (centrilobular hepatocellular hyaline degeneration) and kidneys (nephropathy) of male mice in the 25,000 ppm and 50,000 ppm groups.

Subchronic toxicity of ethylene glycol in Fischer 344/N rats was reported by Melnick *(62*). Groups of 9-10, 7-week-old male and female rats/sex/group were fed diets containing 0, 0.32, 0.63, 1.25, 2.5, or 5.0% ethylene glycol (>99% purity) for 13-weeks. The study authors estimated that the 1.25% concentration was equivalent to 600-1000 mg/kg bw/day in males and the 2.5% concentration was equivalent to 1000-1500 mg/kg bw/day in females. **[Based on the two doses estimated by study authors, CERHR estimated that intakes were approximately 150-250, 300-500, 600-1000, 1200-2000, and 2400-4000 mg/kg bw/day in males and 125-188, 250-375,**

500-750, 1000-1500, and 2000-3000 mg/kg bw/day in females from the low to high dose groups, respectively]. It was determined that ethylene glycol was stable in feed for 2 weeks. Dose selections were based on effects reported in the literature. Parameters evaluated included body and organ weights, blood chemistry, urinalysis, and histopathology. **[There was no discussion of statistical analyses]**. Four males in the 5% group died and bodyweight gain was significantly reduced in males from the 2.5 or 5% groups. Significant organ weight effects included increased relative kidney weights in males and females of the 2.5 and 5.0% groups and decreased relative thymus weight in males of the 5.0% group. Organs were fixed in 10% formalin and a histopathological evaluation was conducted in control and high dose animals, and organs from lower dose groups if gross lesions were observed or if effects were noted in organs of the high dose group. Histopathological observations in kidneys from the 2.5 and 5% group males included toxic nephrosis and deposition of crystals that appeared to be calcium oxalate. Calcium oxalate-like crystals were also detected in the urinary bladder, urethra, and brains of males from the 5% groups. Less severe, multifocal tubular lesions were seen in kidneys of the 5% group females, but there were no oxalate crystals present. No lesions were reported in testes, prostate, ovaries, or uterus. The only treatment-related blood chemistry effects were significantly increased blood urea nitrogen and creatinine levels in males of the 2.5 and 5.0% groups. Treatment had no effect on urinalysis parameters. The authors concluded that ethylene glycol appears to produce no renal toxicity at doses of 1.25% (600-1000 mg/kg bw/day) in males and 2.5% (1000-1500 mg/kg bw/day) in females.

Gaunt et al. *(63*) also examined subchronic toxicity of ethylene glycol exposure in rats. Twenty-five male and female weanling Wistar rats were fed diets containing 0, 0.05, 0.1, 0.25, or 1.0% ethylene glycol (98.5% purity) for 2, 6, or 16 weeks. Doses were equivalent to 0, 35, 71, 180, and 715 mg/kg bw/day in males and 0, 38, 85, 185, and 1128 mg/kg bw/day in females. The aim of dose selection was to obtain no effects at the lower dose levels and renal toxicity at the higher dose levels. **[There was no verification of ethylene glycol levels in food.]** During treatment rats were monitored for clinical signs, food and water intake, weight gain, and renal function. Five rats/sex/group were sacrificed at 2 and 6 weeks and 15 rats/sex/group were sacrificed at 16 weeks. At sacrifice, hematological, serum chemistry, and urinalysis parameters were examined. The kidney, uterus, ovaries, testes, prostate, and seminal vesicles were among the organs that were fixed in 10% neutral buffered formalin, embedded in paraffin wax, and examined histologically in all exposure groups. Methods of statistical analyses, which included Student's t-test and chi-square test were not discussed in detail but were referenced. This summary of results focuses on the group treated for 16 weeks; similar effects were seen in the groups treated for 2 or 6 weeks. There were no clinical signs of toxicity or adverse effects on bodyweight gain, food intake, serum chemistry, or hematology. Significantly increased water intake in females was not dose-related and authors noted that significance likely resulted from low water intake by controls. Tables in the study list the incidence of testicular atrophy and cystic uterus and ovary and there appear to be no changes related to ethylene glycol treatment. **[The table did not report severity of effects]**. The kidney was the only organ with histological changes attributed to ethylene glycol treatment by the authors. Incidence of males with crystals and lesions in the kidney was significantly elevated in the 0.25 and 1.0% dose groups. An increased number of females in the 1.0% group had kidney lesions but statistical significance was not obtained. Excretion of oxalic acid was significantly increased in both sexes in the 1.0% group; in treated males, oxalic acid levels were 100-500% of control levels while the 1.0% females had oxalic acid values that were 30-100% of control values. Also noted in males of the 1.0% group were significant increases in absolute kidney weight, oxalic acid crystals in urine, and secretion of a larger volume of urine with a lower specific gravity. A "no-untoward-effect level" of 0.1% (71 and 85 mg/kg bw/day in males and females, respectively) was identified by study authors.

Robinson et al. *(64)* dosed 10 male and female Sprague Dawley rats/sex/group (85 days old at start of study) with ethylene glycol (100% purity) in drinking water for 10 or 90 days. Only the 90-day portion of this study is described here. Doses in drinking water were 0, 0.25, 0.50, 1.0, or 2.0% in males (205, 407, 947, or 3134 mg/kg bw/day) and 0.50, 1.0, 2.0, or 4.0% in females (597, 1145, 3087, or 5744). Dose selection was based upon results of the 10-day study and concentrations in drinking water were confirmed. All animals were necropsied at the end of exposure and parameters evaluated included hematology and clinical chemistry. A histopathological examination was conducted in kidneys from all animals. For other organs histopathology was examined in 5 control animals per sex and all surviving animals in the high dose group. **[Although a histopathological examination was conducted in male and female reproductive organs from the control and high dose group, the results were not reported].** Statistical analyses included Tukey's multiple comparison procedure, Kruskal-Wallis Rank Sum Test, one-factor ANOVA, Fisher's Exact Test, and Pearson's Correlation Coefficient and Correlation Analysis. Death occurred in 8/10 females in the 4.0% group and 2/10 males in the 2.0% group. Bodyweight gain was significantly reduced in males of the 2.0% group. The only hematological effect was a reduction in leukocyte numbers in females 0.5, 2.0, and 4.0% dose groups. The only significant clinical chemistry findings that appear to be dose-related include increased creatinine levels in males dosed with ≥1% and increased BUN and phosphorus in males of the 2% group. No significant organ weight effects occurred in females. Significant absolute organ weight changes that occurred in males included increased kidney weight at ≥1%, increased brain and gonads weight at 2%, and decreased heart, liver, and lung weight at 2%. **[It does not appear that organ to body weight ratios were statistically analyzed]**. Histopathology was only reported for kidneys. Significant increases in incidence and severity of kidney lesions and and birefringent crystal deposition occurred in the males exposed to ≥1% and females exposed to ≥2%. Lesions in males occurred with greater frequency and severity compared to females.

Blood et al. *(71)* fed two male rhesus monkeys diets containing 0.2% ethylene glycol **[purity not specified]** and one female rhesus monkey a diet containing 0.5% ethylene glycol for three years. **[The ages of the monkeys were not specified and there were no control animals.]** No abnormal calcium deposits were observed in x-rays that were taken every three months during the study. Following sacrifice, a histopathological evaluation **[methods not specified]** was conducted in heart, esophogus, stomach, intestine, liver pancreas, urogenital system (kidneys, ureters, bladder, testes, ovaries, and uterus), spleen, lymph nodes, thyroids, parathyroids, adrenal, pituitary, and bone marrow. The only histopathological effect observed in the kidney of one male was a few scattered glomeruli that were sclerotic and had thickened Bowman's capsules, eosinophilic material in tubules, and mononuclear cells in the interstitium. The authors concluded that no toxic effects were observed in the monkeys.

Histopathological findings in reproductive organs were not reported for a monkey study conducted by Roberts and Seibold *(72)*. Renal toxicity was reported for males exposed to >15 mL/kg bw (17 mg/kg bw) ethylene glycol in drinking water for 6-157 days. The study is limited because only one monkey was exposed for most time durations and some monkeys were exposed to multiple dose concentrations.

2.2.2.2 Inhalation Exposure

Inhalation data are very limited. Because of the scarcity of inhalation data, the Expert Panel evaluated one inhalation study, even though reproductive organs were not examined. Coon et al. *(73*) exposed male and female Sprague-Dawley and Long-Evans rats (n=15/dose), male and female Princeton-derived guinea pigs (n=15/dose), male New Zealand rabbits (n=3/dose), male beagle dogs (n=2/dose) and male squirrel monkeys (n=2/dose) eight hours a day, five days a week for 6 weeks to reagent grade ethylene glycol at concentrations of 0, 10 or 57 mg ethylene glycol/m^3. Continuous 90-day exposures to 12 mg/m^3 were also conducted. Concentrations within exposure chambers were monitored. Serum biochemistry and hematology were examined before and after exposure. Organs that were evaluated histologically in at least half the animals included heart, lung, liver, kidney, and spleen. **[There was no discussion of statistical analyses and it is not known if they were conducted.]** Authors reported normal hematology and blood chemistry values. Mild histopathological changes in liver were sometimes noted in rats, guinea pigs and monkeys but authors did not consider the effects to be treatment related. Eye effects noted with continuous exposure to 12 mg/m^3 included moderate to severe irritation in rabbits and corneal opacity and possible blindness in 2 rats. Deaths were reported for 1 rabbit, 3 guinea pigs, and 1 rat exposed continuously to 12 mg/m^3, but the animals did not display any other types of toxicity.

2.2.2.3 Dermal Exposure

In a prenatal toxicity study, no renal lesions, clinical signs of toxicity, or changes in organ or body weight were observed in CD-1 mice treated dermally with up to 3549 mg/kg bw/day ethylene glycol for 6 hours/day on gd 6-15 *(74*). Complete details of this study are included in Section 3.

2.3 Genetic Toxicity

Because the NTP *(23*) and ATSDR *(5*) already conducted a thorough review of genetic toxicity information, the Expert Panel summarized the main findings of the reviews in Tables 2-9 and 2-10. The majority of findings were negative, but NTP noted positive results in a chromosomal aberration and dominant lethal mutation assay in rats. NTP question the validity of the two positive studies due to limitations such as lack of chemical purity information, no reporting of primary data, and unacceptably low control values. ATSDR stated that, “Because of the information available in *in vitro* culture and animals, it is reasonable to conclude that exposure to ethylene glycol poses minimal risk of causing genotoxic effects in exposed persons."

Table 2-9 *In Vivo* Genotoxicity Results.

Species or Assay Type	Dose	Endpoint	Result	Referenced In
F344 rat	400-1000 mg/kg bw	Dominant Lethal Mutation	Negative	ATSDR *(5)* NTP *(23)*
Rat	120 or 1,200 mg/kg bw	Dominant Lethal Mutation	Positive	NTP *(23)*
Rat	1,200 mg/kg bw	Chromosomal Aberration	Positive	NTP *(23)*
Bracon hebetor (parasitic wasp)	Not specified	Dominant Lethal Mutation	Negative	NTP *(23)*

Table 2-10. *In Vitro* Genotoxicity Results.

Species (strain)	Concentration	Endpoint	Result Without Activation	Result With Activation	Reference
Salmonella typhimurium (Total of five studies)	≤10,000 µg/plate in one study	Mutation	Negative	Negative	ATSDR *(5)* NTP *(23)*
Escherichia coli	NS	DNA Damage	Negative	Negative	ATSDR *(5)* NTP *(23)*
Schizosaccharomyces pombe	NS	Mutation	*	*	ATSDR *(5)* NTP *(23)*
Neurospora crassa	NS	Aneuploidy	*	*	ATSDR *(5)* NTP *(23)*
Mouse lymphoma L5178Y Cells	≤5,000 µg/mL	TFT resistance	Negative	Negative	NTP *(23)*
Chinese Hamster Ovary Cells	≤5,000 µg/mL	Chromosomal Aberration And SCE	Negative	Negative	NTP *(23)*
Human Embryonic Fibroblasts	NS	Chromosomal Aberration	Negative	No Data	NTP *(23)*

*Negative results were obtained but it was not specified if it was in the presence or absence of metabolic activation.
NS=not specified
SCE=Sister chromatid exchange
TFT=Trifluorothymidine

2.4 Carcinogenicity

Human Data

In a study comparing 26 workers with renal cancer to 190 controls in a Texas Chemical plant, no association was found between renal cancer and ethylene glycol exposure *(75)*. ATSDR *(5)* noted that the sample size was small.

Experimental Animal Data

The following two carcinogenicity studies were evaluated in detail since the studies included a histological evaluation of reproductive organs.

The NTP *(23)* conducted a GLP two-year carcinogenicity assay in B6C3F1 mice (age 55-63 days). Sixty animals/sex/group, were exposed to ethylene glycol through diet at doses of 0, 6,250, 12,500, and 25,000 ppm for males, and 0, 12,500, 25,000, and 50,000 ppm for females. Based on measured dietary intakes, the authors estimated these exposure groups to correspond to approximate average doses of 1,500, 3,000, and 6,000 mg/kg bw/day in males, and 3,000, 6,000, and 12,000 mg/kg bw/day in females. Doses for this study were based on results of the NTP 13 week study described under Section 2.2.2.2.1. The parameters observed included survival, body and organ weight, clinical signs, necropsy, hematology, blood chemistry, and histopathology (organs preserved in 10% formalin). Statistical analyses are discussed under the summary for the 13-week study in Section 2.2.2.1. At a 15 month interim sacrifice, 6-10 animals/sex were examined in each group. The number of surviving animals was 23-37/group/sex by the end of the study; there was no difference in survival between treated and control groups. For most organs including reproductive organs, histopathology was examined only in control and high dose groups, in animals that died before study completion, and in tissues with masses or lesions. No significant ($p<0.05$) increases in incidence of neoplastic or nonneoplastic lesions were seen in reproductive organs of treated males (testes, seminal vesicle, prostate, preputial gland, penis, epididymis, ductus deferens, coagulating gland) or females (ovary, oviduct, uterus). The primary effects noted in the 2-year study were: significant hepatocyte hyaline degeneration in the liver of males at the two highest doses (3,000 and 6,000 mg/kg bw/day) and in females at the highest dose (12,000 mg/kg bw/day), and medial hyperplasia of the pulmonary arterioles in females at all doses (3,000, 6,000, and 12,000 mg/kg bw/day). Significant treatment-related nephropathic effects were not observed. A few oxylate-like crystals were seen in renal tubules, urethrae, and/or urinary bladders of high dose males (6,000 mg/kg bw/day). The authors concluded that the study provided no evidence of carcinogenicity in mice.

DePass et al. *(65)* treated groups of 80 male and female Crl: CD-1 mice/sex/group and 130 Fischer 344 rats/sex/group for 2 years with ethylene glycol (99.9% pure) in diet at concentrations resulting in doses of 0, 40, 200 or 1,000 mg/kg bw/day. The doses were based on findings of preliminary studies that demonstrated mild renal toxicity in male rats treated for 40 days with a dose similar to the highest dose in this study. The stability of ethylene glycol in diet was verified. Mice were 42 days old and rats were 38 days old at study initiation. In addition to terminal sacrifice, interim sacrifices were as follows: 20 mice/sex/group at 80 weeks; 10 rats/sex/group at 6 and 12 months, 20 rats/sex/group at 18 months. Observed endpoints in rats included survival, body and organ weight, clinical signs, necropsy, hematology, blood chemistry, and urinalysis. Organs were preserved in 10% formalin and histopathology was conducted in control and high dose animals, all tissue with gross lesions, and target tissues. Reproductive organs examined histologically included ovaries, uterus, epididymides, testes, and prostate. Histopathology was the only parameter examined in mice. Statistical analyses included life-table techniques for

incidence data, and Duncan's and Bartlett's tests, t-test, and ANOVA for continuous data. All male rats in the 1,000 mg/kg bw/day group died or were euthanized before 18 months of treatment. Significant effects in male rats of the high dose group (1000 mg/kg bw/day) at 12 months into the study included decreased body weight gain, increased water intake, increased blood urea nitrogen and creatinine, decreased erythrocytes count, reduced hematocrit, and hemoglobin levels, increased neutrophil numbers, increased urine volume, and decreased urine specific gravity and pH. The authors did not consider changes in any of these parameters to be treatment-related in female rats. Increased kidney weights and urinary calcium oxalate crystals were present in male and female rats of the high dose group. In addition, uric acid crystals in urine were also observed in 1000 mg/kg bw/day females following 18-24 months of treatment. Significant histopathologic lesions in the 1,000 mg/kg bw/day males included tubular dilation, peritubular nephritis, parathyroid hyperplasia, and generalized soft tissue mineralization. Females in the 200 and 1,000 mg/kg bw/day groups experienced fatty changes in liver that reached statistical significance at 1,000 mg/kg bw/day. No treatment-related effects on rat reproductive tissues were reported. There was no evidence of carcinogenicity in rats. No significant increases in treatment-related non-neoplastic lesions were seen in mice. **[Because tables listing histopathological effects in mice were not available, it is not possible to verify a lack of lesions in reproductive organs.]** No evidence for carcinogenicity in mice was found, other than a significant increase in time-adjusted lymphosarcoma in high-dose female mice; the majority of statistical analyses conducted determined that the effect was not statistically significant. The authors concluded that the study provided no evidence of oncogenic effects in rodents.

Health Canada *(9*) considered the DePass et al. *(65*) study to be inadequate for a dose-response assessment due to inconsistent histological assessment of the onset and progression of non-cancer lesions, complete mortality of male rats in the highest dose group, and a dosing regimen that resulted in a 100% lesion incidence at the highest does group (1000 mg/kg bw/day) and minimal effects at the next lower dose (200 mg/kg bw/day). The ACC *(61*) disagreed with the Health Canada evaluation and stated that the DePass study is acceptable for a dose-response assessment. According to the ACC, the two pathologist who reviewed the histology slides at different sacrifice intervals stated that they consulted with each other and used consistent criteria and terminology to assess kidney histology. Furthermore, the ACC noted that the dosing regimen is standard for toxicology studies and the large spacing between doses results in a conservative risk assessment.

ATSDR concluded that "Studies in both humans and animals indicate that there is little carcinogenic risk after ethylene glycol exposure, although the data are scanty."

2.5 Potentially Sensitive Subpopulations

There are no known subpopulations with increased susceptibility to toxicity associated with ethylene glycol exposures. As discussed by ATSDR, *(5*) sensitivity to any chemical exposure could be increased by factors such as compromised organ function, genetic variation, developmental stage, and dietary deficiencies resulting in reduced detoxification and excretion capability. However, none of those factors are known to be specific to ethylene glycol.

ATSDR *(5*) points out that the sweet taste of ethylene glycol, common improper storage and disposal, and undeveloped reasoning skills of children result in a potentially hazardous situation for small children. In 2001, 847 cases of ethylene glycol poisoning were reported in children less than six-years of age *(15*).

2.6 Summary

General Toxicokinetics: Absorption/Distribution/Metabolism/Excretion

Humans can be exposed to ethylene glycol through the oral, dermal, and inhalation routes. Oral intake of ethylene glycol results in rapid and near complete absorption in numerous species including humans, mice, rats, dogs, rabbits, and monkeys, *(5, 24, 30, 31, 32, 35, 36*). In contrast to oral absorption, dermal absorption was found to be slow and incomplete in rats and mice *(30-32*). There are no controlled studies of *in vivo* dermal absorption in humans but one *in vitro* study suggested that absorption of ethylene glycol is slower in human compared to mouse skin *(29*). The study provided only qualitative information due to questionable discrepancies in permeability constants between the control ethanol compound and ethylene glycol in human and mice. For instance, the permeability constant for ethanol was approximately equal in human and mouse skin while the permeability constant for ethylene glycol was 30 to 40 times lower in humans compared to mice. However, the human and animal data set suggest that dermal exposure to ethylene glycol is unlikely to result in human poisonings unless there is an extreme exposure scenario or barrier function is seriously compromised. There are no definitive studies examining absorption of ethylene glycol by the inhalation route in humans. A study in rats suggests that absorption of ethylene glycol is less efficient from the respiratory tract compared to the digestive tract. In the rat study, ~60% of ethylene glycol vapors or mists were deposited largely in the respiratory tract *(37*). Irritating properties of ethylene glycol may limit the amount available for absorption from the respiratory tract due to its warning properties and reflexive decreases in respiratory rate.

Once absorbed through any exposure route, ethylene glycol is readily distributed according to total body water in humans, rats, mice, monkeys and dogs *(5, 24*).

Metabolism of ethylene glycol is qualitatively similar in humans, monkeys, dogs, rabbits, rats, and mice *(5, 23-25*). Figure 2-1 outlines the metabolism of ethylene glycol. In a rate limiting reaction, ethylene glycol is converted to glycolaldehyde by alcohol dehydrogenase. Glycolaldehyde is quickly metabolized to glycolate and to a minor extent glyoxal by cytosolic aldehyde oxidase and aldehyde dehydrogenase (ALDH). Glycolate is a major metabolite that tends to accumulate following high dose ethylene glycol exposure in humans and animals. Human poisoning case studies demonstrated that blood levels of glycolate can exceed levels of ethylene glycol *(39, 40*). Glycolate is converted to glyoxylate by glycolate oxidase or lactate dehydrogenase in the second rate limiting step. Glyoxylate is mainly metabolized to formate and then CO_2. A smaller percent of glyoxylate is metabolized to urinary oxalate and glycine.

Although the general aspects of ethylene glycol metabolism are well characterized, the Panel noted that there remain some questions regarding the more detailed aspects of metabolism. Class I ADH, the primary ADH in human liver, is a dimer composed of randomly associated polypeptide units encoded by 3 loci (ADH1A, ADH1B, and ADH1C). It is not known which of the three forms of human class I ADH is most important in metabolism. In addition, the methods used to study metabolism do not rule out a possible role for CYP2E1. Pyrazole and 4-methylpyrazole, which were used to inhibit ADH activity in metabolic studies, also inhibit CYP2E1. Studies have demonstrated that CYP2E1-generated peroxide could facilitate the non-enzymatic oxidation of ethylene glycol but did not address direct oxidation by CYP2E1 *(38*).

The major elimination products in humans and animals include carbon dioxide in exhaled air and ethylene glycol and glycolate in urine. The half-life for ethylene glycol elimination in humans is 2.5-8.4 hours with little remaining in tissues and urine after 24-48 hours *(5*). In human poisoning cases, treatment with ethanol to prevent additional metabolism prevents a direct comparison of

half-lives in humans versus animals. The normal functioning kidney contributes greatly to the excretion of ethylene glycol in humans with renal half-lives of elimination reported at 15-18 hours *(40, 44*). However rapid renal clearance would not occur in subjects experiencing renal toxicity. In animal studies, elimination half-lives ranged from about 1-3 hours *(30, 33, 35, 36*). Half-lives for both ethylene glycol and glycolic acid were independent of dose in rats, with rapid clearance occurring under saturating conditions *(30-33*), the finding suggests that elimination of parent compound and metabolite is effectively occurring through renal clearance.

Exposure Route and Dose Rate Effects on Metabolic Saturation

A series of studies examined metabolism of ethylene glycol following oral gavage exposure in rats and mice *(30-32*). The studies demonstrated that at low doses, ^{14}C-ethylene glycol is primarily excreted as $^{14}CO_2$ in expired air and to a lower extent, ^{14}C in urine. As doses increase, urinary elimination exceeds excretion in breath. Patterns of parent compound versus metabolites in urine also change according to dose with percent urinary glycolate increasing according to dose. The changes in excretion pattern suggest saturation of metabolism that occur at doses between 10 to 100 mg/kg bw/day in mice, 10 to 400 mg/kg bw in female rats, and doses exceeding 1000 mg/kg bw in male rats. Pottenger et al. *(33*) also observed indications of saturated metabolism in female rats, as noted by elevations in total urinary elimination (excretion in breath not measured) and percent urinary glycolic acid excretion with increasing dose. The shift in metabolism was seen at doses between 150-500 mg/kg bw. The Panel noted that half-lives of ethylene glycol and glycolic acid reported by Pottenger et al. *(33*) were independent of concentration and thus incompatible with a zero order process. However the Panel stated that the beta-elimination rate calculations may have been insensitive to identifying saturating conditions due to possible rapid elimination by other pathways (e.g., renal elimination) and several key glycolic acid data points that were at or near the detection limit. Together the data from Frantz et al. *(30-32*) and Pottenger et al. *(33*) suggest that saturation of ethylene glycol metabolism and potential glycolate accumulation in blood occur between doses of 150-500 mg/kg bw in rats.

Frantz et al. *(30-32*) also studied excretion patterns in mice and rats exposed dermally to neat or 50% aqueous ^{14}C-ethylene glycol. Dermal exposure to 10 or 1000 mg/kg bw/day resulted in excretion occurring primarily through $^{14}CO_2$ in breath and secondarily through ^{14}C in urine. Dermal exposure to 1000 versus 10 mg/kg bw did not appear to increase ethylene glycol or glycolate levels in urine and the majority of ^{14}C was found as unmetabolized ethylene glycol. The finding suggests that in rats, removal of glycolic acid is efficient following dermal exposure with up to 1000 mg/kg bw/day for six hours and that saturation of metabolic enzymes does not occur under those conditions.

Excretion occurring primarily through $^{14}CO_2$ exhalation and secondarily through urinary ^{14}C excretion suggested lack of metabolic saturation in rats inhaling ethylene glycol at 184 mg/m^3 (7.1 mg/kg bw) as aerosol for 17 minutes or 32 mg/m^3 (1.25 mg/kg bw) as vapors for 30 minutes *(37*). Exposure concentrations were based on aerosol levels found to be irritating in humans *(26*) and the observation that 20% of total ethylene glycol is present as vapor during the generation of an aerosol. The study demonstrates that exposure to an irritating concentration of ethylene glycol will likely result in a systemic dose that is well below oral doses resulting in metabolic saturation and glycolate accumulation. Therefore the warning properties of ethylene glycol should prevent exposures resulting in metabolic saturation.

Carney et al. *(42*) measured ethylene glycol and glycolate levels in the blood of rats exposed to 1,000-2,000 mg/kg bw/day ethylene glycol by bolus sc injection or slow, continuous sc infusion. Blood ethylene glycol and glycolate levels were lower following sc infusion versus bolus dosing,

but infusion did lead to a ten-fold increase in glycolate plasma levels at 2000 mg/kg bw/day. It therefore appears that saturation occurred somewhere between 1000 and 2000 mg/kg bw/day with continuous dosing. In contrast Pottenger et al. *(33*) reported saturation between 150-500 mg/kg bw/day following bolus gavage dosing. Therefore, it appears that continuous dosing is 3-10-fold less efficient at saturating glycolate metabolism than bolus dosing. The Expert Panel noted that because AUC was not determined in this study, it is not known if bioavailability varied between the two dosing methods.

Toxicokinetic and Metabolic Issues Related to Pregnancy

The rat study conducted by Pottenger et al. *(33*) examined two issues related to pregnancy: (1) possible changes in toxicokinetics and metabolism of ethylene glycol related to pregnancy and (2) if there is evidence of metabolic saturation at doses that produce teratogenicity in rodents (see Section 3). The study compared toxicokinetic parameters and excretion patterns in pregnant (gd 10-11) versus non-pregnant rats and found no significant differences between the two groups. As noted by the Panel, gd 10-11 represents a sensitive time period for ethylene glycol-induced developmental toxicity, but is limited to a narrow window of gestation. It is not known if physiological changes that occur in later stages of pregnancy would have resulted in significantly different toxicokinetic results. In addition, extrapolation of the findings to humans is unclear due to differences in ontological developmental of ADH enzymes between rats and humans and uncertainty regarding specific human enzymes that metabolize ethylene glycol. Such factors lend uncertainty to the extrapolation of rat maternal toxicokinetic data to estimate dosimetry in the human fetus.

As discussed above, the Panel noted evidence of metabolic saturation at doses between 150 to 500 mg/kg bw/day in the Pottenger et al. *(33*) study. Developmental toxicity studies conducted in rats (see Section 3) identify a NOEL of 500 mg/kg bw/day and LOEL of 1000 mg/kg bw/day, thus suggesting that developmental toxicity occurs under conditions resulting in saturation of glycolate removal.

Metabolic capacity of ADH and ALDH enzymes in placenta was studied in humans and rats using ethanol as a substrate *(48, 49, 50*). The studies found that isoenzymes in placenta of rats and humans had very low activity, thus suggesting the placenta has limited capacity to metabolize ethylene glycol and its metabolites.

Development of ADH and ALDH

ADH activity in livers of fetuses, children, and adults has been compared using ethanol as a substrate. One study examined ADH activity in livers from 56 fetuses, 37 premature infants, and 129 adults, and reported that ADH activity was 30% of adult activity in 9-22 week old fetuses and 50% of adult activity in infants under 1 year of age, including premature infants *(52*). Qualitatively similar results were obtained in a second study using1-3 liver samples/age group; the study reported that ADH reached adult activity levels at 5 years of age *(51*). Similar patterns of development were seen for ADH and ALDH enzymes in rat studies *(50*). Ontogeny of ADH class I isoforms in humans was found to change according to developmental stage *(52*). In fetuses with a mean gestational age of 11 weeks, only the ADH1A enzyme was detected from liver samples. As gestational age advanced, expression of the ADH1B and ADH1C enzymes began to increase, with the two isoforms first detected at 17 and 19 weeks, respectively. In adult liver samples, ADH1B and ADH1C expression were equal and expression of ADH1A was not detectable.

The Expert Panel noted that extrapolation of the ethanol metabolism data is uncertain due to the paucity of knowledge regarding isoform specificity towards ethylene glycol. If it is assumed that the enzyme most active in ethanol metabolism, ADH1B, is also most active in ethylene glycol metabolism, than it would be predicted that significant fetal metabolism would not occur until 20-36 weeks of gestation.

Genetic Polymorphisms

Polymorphisms resulting in altered phenotypes are observed in the ADH1B (ADH1B*2 and ADH1B*3) and ADH1C (ADH1C*2) loci *(55, 56, 57, 58*). The ADH1B*2 allele occurs in an estimated 15% of Caucasians, 85% of Asians, and less than 5% of African Americans. An estimated 15% of African Americans have the ADH1B*3 allele, which is virtually absent in other ethnic groups. Ethanol V_{max} values for the ADH1B*2 and ADH1B*3 enzymes are 100-fold higher than the value reported for the ADH1B*1 enzyme. Ethanol affinities for ADH1B*2 and ADH1B*3 are about 20- and 70-fold lower than the affinity for ADH1B*1, respectively.

ALDH2 (also called E_2, ALDHI, or $ALDH_2$) and ALDH1 (also called E_1, ALDHII, or $ALDH_1$) are the primary ALDH isoenzymes in human liver *(55, 56, 57*). About 50% of Asians carry a phenotypically null variant of the ALDH2 enzyme.

General Toxicity

Human Data

Acute effects associated with ingestion of ethylene glycol are well characterized *(5, 23, 24, 59*). CNS depression and gastrointestinal upset are common symptoms occurring within 30 minutes to 12 hours following ingestion. Metabolic acidosis can occur following that time period and is characterized by reduced blood pH and bicarbonate levels; serum hyperosmolality and increased anionic gap can also occur. Metabolic acidosis is possibly the cause of cardiopulmonary and renal toxicity that are often observed with ethylene glycol poisonings. Renal toxicity is characterized by oxalate crystal deposition and tubular necrosis. Symptoms associated with renal toxicity include polyuria followed by oliguria and flank pain. Calcium oxalate deposition is thought to be a major factor in renal failure. The human lethal oral dose has been estimated at 1400-1600 mg/kg bw but there is considerable uncertainty associated with that estimate since human intake is difficult to quantify. In survivors of ethylene glycol poisoning, neurological symptoms possibly involving cranial nerves are infrequently seen six or more days following exposure.

One study examined acute exposure to ethylene glycol mist and found that 140 mg/m^3 caused nasal and throat irritation while levels above 200 mg/m^3 were intolerable *(26*). It has been noted that dermal exposure to ethylene glycol is not likely to result in toxicity *(5*). Ethylene glycol appears to be a mild skin irritant but not sensitizer.

There is a limited amount of data on repeated human exposures to ethylene glycol. In a controlled study where 19 men were continuously exposed to 3-67 mg/m^3 ethylene glycol for 20-22 hours/day for 30 days, ethylene glycol levels in blood and urine were similar to 10 unexposed controls, *(26*) there were no effects on heart, brain, or neurobehavioral function, urinalysis, hematological, or blood chemistry parameters (including urea nitrogen, creatinine, and plasma pH). A study of 33 male aviation workers exposed to <2.5-22 mg/m^3 and <17-190 mg/m^3 ethylene glycol mists and vapors, respectively, over a three month period found no evidence of ethylene glycol-induced acute or chronic renal toxicity (measured by urinary β-N-acetyl-

glucosaminidase, albumin, β-2-microglobulin, and retinol-binding protein) *(19*); however, the study authors noted that the study may have had limited statistical power due to small sample size and wide variations in renal function parameters. Compared to unexposed controls, ten males had higher levels of ethylene glycol in urine and a significant reduction in urinary glycosaminoglycans level and increase in urinary ammonia *(20*). Slight but not significant increases in urinary oxalic acid levels and decreases in urinary calcium concentration and succinate dehydrogenase activity were also noted for the exposed mechanics.

Experimental Animal Studies

Toxic effects observed in acute or repeat dose studies in animals are similar to those observed in humans and include central nervous system effects followed by metabolic acidosis, cardiopulmonary effects and then renal toxicity *(5, 24, 59*). Such symptoms have been observed in rats, mice, guinea pigs, rabbits, dogs, cats, and non-human primates. Table 2-7 outlines MLDs and LD50s observed in various animal species.

Systemic effects observed in key repeat dose chronic and subchronic rodent studies are outlined in Table 2-11. In repeat-dose rodent subchronic and chronic toxicity studies, the kidney was consistently shown to be a target of ethylene glycol toxicity. Exposure to high concentrations of ethylene glycol was shown to produce kidney lesions, oxalate crystal deposition, and other indications of renal toxicity such as increased BUN and creatinine levels. Renal toxicity was found to vary according to sex and species. In dietary or drinking water exposure studies conducted for 13-16 weeks, renal crystal deposition and/or lesions were noted in male rats treated with ≥180-2000 mg/kg bw/day ethylene glycol *(62-64*) in those same studies kidney effects in female rats were less severe and occurred at higher dose levels (1128-3000 mg/kg bw/day). In a two-year dietary study in rats, exposure to 1000 mg/kg bw/day ethylene glycol resulted in renal oxalate crystal deposition in males and females with males also experiencing increases in blood urea nitrogen, severe lesions, and death *(65*). Mice were found to be less sensitive to ethylene-glycol induced renal toxicity. In a 13 week and 2-year dietary study, mild renal nephropathy or a few oxalate-like crystals were observed in males at doses of 6000-8700 mg/kg bw/day ethylene glycol; *(23, 62*) in those same studies, no renal crystals or lesions were noted in female mice at doses ≥12,000 mg/kg bw/day. Renal toxicity was reported in monkeys given ethylene glycol in drinking water but dose limitations preclude the identification of an effect concentration *(72*). Oxalate crystal deposition and a 40% death rate was noted in pregnant rabbits gavage-dosed with 2000 mg/kg bw/day ethylene glycol on gd 6-19, *(68*) while no overt toxicity was noted in rats or mice gavage dosed with 5000 and 3000 mg/kg bw/day, respectively, during gestation *(69*). Therefore, it appears that rabbits are affected differently by ethylene glycol than rats or mice. As noted in Table 2-11, liver effects were also noted in the rodent studies but not as consistently as kidney effects.

An inhalation study examined ethylene glycol toxicity in rats, guinea pigs, rabbits, monkeys, and dogs *(73*) but was limited by insufficient reporting of protocol details and results.

Genetic Toxicity

Negative results were reported for the majority of *in vivo* and *in vitro* genetic toxicity tests that examined dominant lethal mutations, chromosomal aberrations, mutations, aneuploidy, and DNA damage *(5, 23*). Positive results were obtained in an *in vivo* chromosomal aberration assay and dominant lethal mutation assay in rats, but the NTP questioned the validity of the studies based on study limitations *(23*). ATSDR stated that, "Because of the information available in *in vitro* culture and animals, it is reasonable to conclude that exposure to ethylene glycol poses minimal

risk of causing genotoxic effects in exposed persons."

Carcinogenicity

A limited human study found no association between renal cancer and ethylene glycol exposure in 26 workers and 190 controls from a Texas chemical plant *(5, 75*).

Two chronic cancer studies in mice and one in rats found no evidence of carcinogenicity following dietary exposure to up to 6,000 mg/kg bw/day in male mice, 12,000 mg/kg bw/day in female mice, and 1000 mg/kg bw/day in male and female rats for 2 years *(23, 65*).

Table 2-11. Summary of Key Rodent Subchronic and Chronic Toxicity Studies.

Dose (mg/kg bw/day	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
M: 830-1110, 1630-2190, 3230-4350, 6450-8700, 12,900-17,400[a] **F:** 1020-1420, 2020-2800, 4000-5560, 8000-11,110, 16,000-22,220 [a]	13-week dietary exposure	B6C3F1	**M – 6450-8700:** Mild liver lesions and nephropathy **M - 12,900-17400:** Mild liver lesions and nephropathy No effects on survival, body or organ weight, hematology, blood chemistry urinalysis, or reproductive organ histopathology.	Melnick et al. *(62)* NTP *(23)*
M: 150-250, 300-500, 600-1000, 1200-2000, 2400-4000 **F:** 125-188, 250-375, 500-750, 1000-1500, 2000-3000	13-week dietary exposure	Fischer 344/N rat	**M – 1200-2000:** ↓BW gain, ↑Relative kidney weight, renal toxic nephrosis and crystal deposition , ↑BUN and creatinine **M – 2400-4000:** Death in 4 M, ↓BW gain , ↑Relative kidney weight, ↓Relative thymus weight, renal toxic nephrosis and crystal deposition, crystals in bladder urethra and brain, ↑BUN and creatinine **F – 1000-1500:** ↑Relative kidney weight **F – 2000-3000:** ↑Relative kidney weight, renal lesions but no crystal deposition No histopathology in reproductive organs	Melnick et al. *(62)* NTP *(23)*
M: 35, 71, 180, 715 **F:** 38, 85, 185, 1128	16-week dietary exposure	Wistar rat	**M – 180:** ↑Renal lesions and crystal deposition **M – 715:** ↑Renal lesions and crystal deposition, ↑oxalate excretion and crystals In urine, ↑kidney weight, ↑urine volume, ↓ urine specific gravity **F – 1128:** Non-significant ↑ in renal lesions, ↑oxalate excretion No treatment-related reproductive organ histopathology, bodyweight gain, or hematology	Gaunt et al. *(63)*
M: 205, 407, 947, 3134 **F:** 597, 1145, 3087, 5744	90-day drinking water exposure	Sprague - Dawley Rat	**M – 947:** ↑creatinine, ↑kidney weight, ↑renal lesions and crystal deposition **M - 3134:** Death in 2/10, ↓BW gain, ↑creatinine, ↑BUN and phosphorus, ↑kidney, brain and gonad weight, ↓heart, liver, and lung weight, ↑renal lesions and crystal deposition **F – 597:** ↓Leukocyte numbers **F – 3087:** ↓Leukocyte numbers, ↑renal lesions and crystal deposition **F - 5744:** Death in 8/10, ↓Leukocyte numbers, ↑renal lesions and crystal deposition	Robinson et al. *(64)*
M: 1500, 3000, 6000 **F:** 3000, 6000,	2-year dietary study	B6C3F1	**M –** 3000: ↑Liver lesions **M –** 6000: ↑Liver lesions, few oxalate-like crystals	NTP *(23)*

Dose (mg/kg bw/day	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
12,000			**F –** 3000: ↑Arteriole hyperplasia **F –** 6000: ↑Arteriole hyperplasia **F –** 12,000: ↑Liver lesions, ↑arteriole hyperplasia No neoplastic or nonneoplastic lesions in kidney or reproductive organs. No effects on survival, bodyweight gain, hematology, or blood chemistry.	
40 200 1000	2-year dietary study	CD-1 mice	No treatment-related histopathology	DePass et al. *(65)*
40 200 1000	2-year dietary study	Fischer 344 Rats	**M - 1000:** ↑ Death (M), ↓BW gain, ↑water intake, ↑BUN and creatinine, ↓erythrocytes, hematocrit, and hemoglobin, ↑neutrophils, ↑urine volume, ↓urine specific gravity and pH, ↑kidney weight, ↑urinary oxalate crystals, ↑renal lesions **F – 1000:** ↑Kidney weight, ↑urinary oxalate crystals, ↑uric acid crystals, ↑liver fatty changes No lesions in reproductive system and no evidence of carcinogenicity.	DePass et al. *(65)*

[a]Doses estimated by CERHR

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

No human developmental toxicity data were identified.

3.2 Experimental Animal Data

3.2.1 Oral Exposure

3.2.1.1 Prenatal Toxicity Studies

3.2.1.1.1 Mouse

Price et al. *(69)* looked at prenatal developmental toxicity associated with oral exposure to ethylene glycol in COBS Crl:CD-1 mice in a study conducted according to Good Laboratory Practices (GLP). On gd 6-15, timed mated mice received gavage doses of ethylene glycol (>99% purity) in distilled water at 0, 750, 1500, or 3000 mg/kg bw/day. Doses were based on results of preliminary experiments and dosing solutions were verified to be within 10% of theoretical concentrations. At scheduled sacrifice on gestation day (gd) 17, confirmed pregnant females, 23-25 per group, were evaluated. The gravid uterus was weighed and the status of uterine implants recorded. Each live fetus was examined for external, visceral and skeletal malformations. Viscera were examined using the Staples fresh tissue dissection technique and the skeleton was examined by staining with Alizarin Red S stain. The heads of half the fetuses were fixed in Bouin's solution and examined. Statistical analyses were conducted by the General Linear Model procedure and included tests for linear trends, analysis of variance (ANOVA), Williams Multiple Comparison Test, Dunnett's Test, Chi-Square test, or Fisher Exact Probability Test.

Incidences for statistically significant findings in the Price et al. *(69)* study are summarized in Table 3-1. The adjusted maternal weight gain (excluding gravid uterus weight) was not different from the controls in any treated group, but weight gain during treatment and gestation was significantly reduced in the 1500 and 3000 mg/kg bw/day groups. Absolute but not relative liver weight was decreased in the 1500 and 3000 mg/kg bw/day dose groups; there was no histological evaluation. The percent non-live implants per litter exhibited a dose related increase; however, no dose was significantly different from controls in pair wise comparisons. A significant reduction in live fetuses/litter was noted in the 3000 mg/kg bw/day group. Fetal weight was significantly decreased at all doses. The proportion of live litters with malformed live fetuses and the percentage of malformed fetuses per litter was significantly greater than controls in all treated groups. **[This was the only malformation data presented as fetuses affected per litter.]** The number of litters containing fetuses with external and visceral malformations was significantly increased at the 3000 mg/kg bw/day dose while numbers of litters containing fetuses with skeletal malformations were significantly increased at all dose levels. The most common malformations involved neural tube closure defects and craniofacial and axial skeletal dysmorphogenesis. The study authors concluded that severe developmental toxicity occurred at doses that did not produce serious maternal toxicity. **[The Expert Panel concluded that a developmental toxicity NOAEL was not established in this study.]**

Strengths/Weaknesses: This study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. Its primary shortcomings are the use of high dose levels and the resultant outcome that compound-related effects were observed in offspring at all dose levels, prohibiting the establishment of a NOAEL for effects in offspring.

Utility (Adequacy) for CERHR Evaluation Process: While this study is not useful for risk extrapolation purposes, it does provide background regarding the types of developmental effects that may be observed at high to very high doses. It is noted that gavage administration delivers high amounts of agent over a brief time period, which is unlikely to mirror expected human exposure.

Table 3-1. Prenatal Toxicity Study of Ethylene Glycol in Mice by Price et al. *(69)*.

Effect	**Doses (mg/kg bw/day)**			
	0	**750**	**1500**	**3000**
Maternal bodyweight gain (gd 6-15; g)	12.40	11.58	8.54**	8.42**
Maternal liver weight (g)	2.72	2.63	2.49**	2.47**
No. Live fetuses/litter	11.88	11.50	10.41	9.83***
Fetal bodyweight/litter (g)	0.974	0.882**	0.787**	0.712**
% Live malformed fetuses/litter	0.25	10.00**	37.77**	56.54**
No. litters with malformed fetuses/no. examined (%)	1/25 (4.00)	16/24* (66.67*)	18/22* (81.82*)	22/23* (95.65*)
No. litters with external malformations/no. examined [%]	0/25 [0]	3/24 [12.50]	2/22 [9.09]	8/23** [34.78]
No. litters with visceral malformations/no. examined [%]	0/25 [0]	0/24 [0]	2/22 [9.09]	7/23** [30.4]
No. litters with skeletal malformations/no. examined [%]	1/25 [4.00]	15/24* [62.50]	17/22* [77.27]	22/23* [95.65]

Protocol: CD-1 mice were exposed to ethylene glycol by gavage on gd 6-15. Dams were sacrificed on gd 17 and fetuses from 22-25 litters/group were evaluated for prenatal developmental toxicity.
Notes:
*=p<0.001, **=p<0.01, ***=p<0.05
( )=Values calculated by authors; [ ]=Values calculated by CERHR
There were no effects on corrected maternal weight gain.

Tyl and Frank *(76)* studied the effects of oral exposure to ethylene glycol on mouse prenatal developmental toxicity in a study conducted according to GLP. Timed-pregnant Crl:CD-1 (ICR) BR mice (30/dose group) received a daily gavage dose of ethylene glycol (100% purity) in deionized water at 0, 50, 150, 500 or 1500 mg/kg bw/day on gd 6-15. Doses were selected to be at or below the mid and low dose levels of the Price et al. *(69)* study. Concentrations of dosing solutions were verified. At scheduled sacrifice on gd 18, the adult mice (19-24/treatment group) were weighed and kidneys were retained for subsequent microscopic examination. The gravid uterus was weighed and examined and the status of uterine implants recorded. Each live fetus was examined for external, visceral, and skeletal malformations. Visceral effects were evaluated using the Staples method and skeletal effects were examined by staining with alizarin red S. The litter was the statistical unit of comparison. Statistical analyses for continuous variables included Levene's test for equal variances, ANOVA, or t-tests with Bonferroni probabilities. Nonparametric data were evaluated with the Kruskal-Wallis test followed by the Mann-Whitney U test and incidence data were compared with Fisher's Exact Test. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

Incidences of statistically significant effects observed in the Tyl and Frank *(76)* study are listed in Table 3-2. No chemical-related maternal toxicity, including effects on bodyweight, water intake, and liver and kidney weight, was observed at any dose level. No significant effects were noted on the number of corpora lutea per dam or the number of total nonviable or viable implants per litter. Fetal body weights per litter were significantly reduced at the 1500 mg/kg dose group. There were no significant increases in the incidence of individual or total external or visceral malformations. The incidence of total malformations in litters was significantly increased in the 500 mg/kg bw/day group, but no individual type of malformation was reported to be statistically significant at that dose level. Total skeletal malformations were significantly increased in litters of the 1500 mg/kg bw/day dose groups. Skeletal malformations included fused or extra ribs and fused thoracic or lumbar arches. The incidences of one individual skeletal variation (extra lumbar rib) in litters from the 500 mg/kg bw/day group and 23 individual skeletal variations (i.e., poorly ossified thoracic and lumbar centra, extra lumbar ribs…) in litters of the 1500 mg/kg bw/day group were significantly increased; total skeletal variations were not significantly increased in any dose group. The study authors identified a maternal and fetal NOEL of 1500 and 150 mg/kg bw/day, respectively, under the conditions of this study. **[The Expert Panel agreed with the authors interpretation.]**

Strengths/Weaknesses: This study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. The doses selected included doses below those reported by Price et al. *(69)* permitting the identification of a NOAEL for effects in offspring following gavage exposure.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which is unlikely to mirror expected human exposure.

Table 3-2. Developmental Toxicity Study of Ethylene Glycol in CD Mice by Tyl and Frank *(76)*.

Effect	Doses (mg/kg bw/day)				
	0	**50**	**150**	**500**	**1500**
Fetal bodyweight per litter (gd 6-15; g)	1.325	1.369	1.330	1.285	1.156**
No. litters with skeletal malformations/no. examined (%)	2/19 (10.5)	3/20 (15.0)	1/24 (4.2)	5/24 (20.8)	17/21** (81.0)
No. litters with malformations/ no. examined (%)	3/19 (15.8)	7/20 (35.0)	5/24 (20.8)	12/24*** (50.0)	17/21** (81.0)
Skeletal variations				[a]	[a]
NOELs			Fetal		Maternal

Protocol: CD-1 mice were exposed to ethylene glycol by gavage on gd 6-15. Dams were sacrificed on gd 18 and fetuses from 19-24 litters/group were evaluated for prenatal developmental toxicity.
Notes:
*=p<0.001, **=p<0.01, ***=p<0.05
[a]See text for description of statistically significant increases in variations.
There were no effects on maternal weight gain, water intake, liver or kidney weight, number of corpora lutea and implantation sites, and external or visceral malformations.

3.2.1.1.2 Rat

Price et al. *(69)* examined rat prenatal toxicity in a study conducted according to GLP. Timed-pregnant CD rats were dosed by gavage with ethylene glycol (>99% purity) in distilled water at 0, 1250, 2500, or 5000 mg/kg bw/day on gd 6-15. Doses were based on results of preliminary experiments and dosing solutions were verified to be within 10% of theoretical concentrations. At scheduled sacrifice on gd 20, maternal liver weight, kidney weights and gravid uterus weight were determined for each dose group (27-29 rats/group). Number of implantation sites, resorptions, dead fetuses and live fetuses were recorded. Each live fetus was weighed, and examined for external, visceral and skeletal defects. Viscera were examined using the Staples fresh tissue dissection technique and the skeleton was examined by staining with Alizarin Red S stain. The heads of half the fetuses were fixed in Bouin's solution and examined. Statistical analyses were conducted by the General Linear Model procedure and included tests for linear trends, analysis of variance (ANOVA), Williams Multiple Comparison Test, Dunnett's Test, Chi-Square test, or Fisher Exact Probability Test.

Incidences for statistically significant findings in the Price et al. *(69)* study are summarized in Table 3-3. No clinical signs were observed in the treated pregnant rats except for piloerection. No effect on corrected maternal weight gain (excluding gravid uterus weight) was observed (data not shown in Table 3-3); however, the body weight gains among all treated groups were significantly reduced during the treatment period. Body weight gains for the 2500 and 5000 mg/kg/day groups were significantly reduced for the entire gestational period (data not shown in Table 3-3). Maternal water consumption was increased throughout the treatment and post treatment period in a dose related manner with significantly more water consumed in the 2500

and 5000 mg/kg bw/day groups. The only organ weight effects were significantly decreased absolute liver weight in the 5000 mg/kg bw/day group and increased relative kidney weight in the 2500 and 5000 mg/kg bw/day groups; no histopathology was conducted. A statistically significant increase in post implantation loss was observed in the 5000 mg/kg bw/day group. Live litter size was significantly reduced at the 2500 and 5000 mg/kg bw/day dose levels. Fetal body weight was decreased at these same dose levels. There was a significant increase in the percentage of fetuses malformed per litter at the 2500 and 5000 mg/kg bw/day dose level. **[This was the only malformation data presented as fetuses affected per litter.]** A significant increase in the percentage of litters with malformed fetuses was observed in all treated groups. Significant increases were noted for the number of litters containing fetuses with external malformations (5000 mg/kg bw/day), visceral malformations (1250 and 5000 mg/kg bw/day), and skeletal malformations (2500 and 5000 mg/kg bw/day). The most common malformations were neural tube closure defects and craniofacial and axial skeletal dysmorphogenesis. The study authors concluded that severe developmental toxicity occurred at doses that did not produce serious maternal toxicity. **[The Expert Panel noted that the claim for increased visceral malformations in the 1250 mg/kg/day was based on 7 cases of hydroureter, 3 cases of hydronephrosis, and 2 great artery anomalies. The Panel disagreed with the classification of these effects as malformations and stated that the effects should be classified as variations. In addition, none of the findings were repeated in a subsequent study by Neeper-Bradley et al. *(77)* described below. The Expert Panel concluded that the data in this study appear to support a NOAEL of 1250 mg/kg bw/day; however, the ensuing study by Neeper-Bradley et al. *(77)* identified a developmental NOEL of 500 mg/kg/day.]**

Strengths/Weaknesses: This study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. The day of sacrifice (gestational day 20) is earlier than that of the ensuing studies, which used gestational day 21. This makes direct comparison of some of the data difficult.

Utility (Adequacy) for CERHR Evaluation Process: This study could be useful for risk extrapolation purposes, with the caveats that (1) exposures were delivered as bolus doses by means of gavage administration, which is unlikely to mirror expected human exposure; and (2) ensuing studies established a lower NOEL.

Table 3-3. Developmental Toxicity Study of Ethylene Glycol in CD Rats by Price et al. *(69)*.

Effect	Doses (mg/kg bw/day)			
	0	**1250**	**2500**	**5000**
Maternal bodyweight gain (gd 6-15; g)	42.03 ± 1.96	34.81 ± 1.73**	29.45 ± 1.38**	20.68 ± 1.93**
Maternal liver weight (g)	15.47 ± 0.26	15.01 ± 0.28	14.95 ± 0.27	13.70 ± 0.35***
Relative maternal kidney weight (% bodyweight)	0.517 ± 0.012	0.531 ± 0.007	0.573 ± 0.008**	0.615 ± 0.021**
Maternal water consumption (gd 6-15; g)	130.3 ± 4.3	128.4 ± 3.0	154.4 ± 4.0**	165.0 ± 3.8**
% Postimplantation loss/litter	4.70 ± 1.23	6.35 ± 1.85	6.27 ± 1.35	21.34 ± 5.24***
No. live fetuses/litter	13.54 ± 0.28	12.75 ± 0.38	11.90 ± 0.60***	11.04 ± 0.79***
Fetal bodyweight/litter (g)	3.404 ± 0.052	3.312 ± 0.058	2.916 ± 0.056**	2.388 ± 0.089**
% Live fetuses malformed/litter	1.37 ± 0.97	6.65 ± 2.04	25.11 ± 4.84**	73.53 ± 6.42**
No. litters with malformed live fetuses/no. examined [%]	2/28 (7.14)	11/28** (39.29**)	20/29* (68.97*)	25/26* (96.15*)
No. litters with external malformations/no. examined [%]	0/28 [0]	0/28 [0]	4/29 [13.79]	15/26* [57.69]
No. litters with visceral malformations/no. examined [%]	0/28 [0]	6/28*** [21.43]	2/29 [6.90]	8/26** [30.77]
No. litters with skeletal malformations/no. examined [%]	2/28 [7.14]	6/28 [21.43]	19/29* [65.52]	24/26* [92.31]

Protocol: CD rats were exposed to ethylene glycol by gavage on gd 6-15. Dams were sacrificed on gd 20 and fetuses from 26-29 litters/group were evaluated for prenatal developmental toxicity.
Notes:
*=p<0.001, **=p<0.01, ***=p<0.05
( )=Values calculated by authors
[ ]=Values calculated by CERHR
There were no effects on maternal corrected weight gain or clinical signs.

Neeper-Bradley et al. *(77, 78)* investigated the prenatal developmental toxicity of ethylene glycol administered by gavage to pregnant rats in a study conducted according to GLP. On gd 6-15, timed-pregnant Crl:CD (Sprague-Dawley) rats (25/dose group) received daily doses of ethylene glycol (99.9% purity) in deionized water at 0, 150, 500, 1000, or 2500 mg/kg bw/day. Concentrations of dose solutions were analytically verified. **[No rationale was provided for**

dose selection.] At scheduled sacrifice on gd 21, 22-25 dams/group were evaluated for body, liver, and kidney weight. The gravid uterus was weighed and examined for status of implantation sites. A total of 21-24 litters/group were examined. Live fetuses were weighed, sexed and examined for external abnormalities. All fetuses were examined for visceral malformations by the Staple's method and for skeletal malformations and variations by staining with alizarin red S. Heads from one half of the fetuses were fixed in Bouin's solution and examined for soft tissue malformations. The litter was the unit of comparison in statistical analyses. Continuous variables were analyzed by Levene's test for equal variances, ANOVA, and/or t-tests with Bonferroni probabilities. Non-parametric data were analyzed by the Kruskal-Wallis test, Mann-Whitney U test and/or Fisher's Exact Test. For all statistical tests, a probability value of $P < 0.05$ (two-tailed) was used as the critical level of significance.

Incidences of statistically significant findings are outlined in Table 3-4. No treatment-related maternal deaths, abortions, or early deliveries occurred. Maternal weight gain was significantly decreased in the 2500 mg/kg bw/day group, but corrected weight gain was unaffected. Maternal water consumption significantly increased among rats receiving 2500 mg/kg bw/day. Significant organ weight changes included increased relative liver weight at 1000 and 2500 mg/kg bw/day and increased relative and absolute kidney weight at 2500 mg/kg bw/day. Microscopic evaluation of kidneys from the high dose dams revealed no treatment-related lesions; livers were not examined. There were no observed effects on gestational parameters that included number of corpora lutea, total number of implantations/litter, or on sex ratio. Significant developmental effects in the 1000 mg/kg bw/day dose group included reduced fetal body weight and increased incidences of litters containing fetuses with two skeletal malformations (missing thoracic arch and missing ribs). At 2500 mg/kg bw/day, significantly increased frequencies of litters containing fetuses with visceral, skeletal, external, and total malformations were observed. Defects observed in the high dose group included gastroschisis, hydrocephaly, lateral ventricle dilation, umbilical hernia, and malformations of the ribs and vertebrae. A significantly increased incidence of skeletal variants (primarily involving delayed ossification) were also observed in litters from the 1000 and 2500 dose groups. The author reported NOELs for maternal and developmental toxicity were 1000 and 500 mg/kg bw/day, respectively. **[The Expert Panel concurs with the authors' selection of a NOEL under the conditions of this study.]**

Strengths/Weaknesses: This study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. The day of termination (gestational day 21) is later than that of the previous studies, which used gestational day 20. This makes direct comparison of some of the data difficult.

Utility (Adequacy) for CERHR Evaluation Process: This study should be useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which is unlikely to mirror expected human exposure.

Table 3-4. Prenatal Toxicity Study of Ethylene Glycol in CD Rats by Neeper-Bradley et al. *(77, 78)*.

Effect	Doses (mg/kg bw/day)				
	0	**150**	**500**	**1000**	**2500**
Maternal bodyweight gain (gd 6-15; g)	35.53 ± 12.2	40.79 ± 8.6	40.08 ± 9.1	39.23 ± 6.4	26.46 ± 11.3**
Maternal water intake (gd 6-15; g/rat/day)	34.02 ± 4.4	34.32 ± 4.7	36.38 ± 10.7	34.87 ± 6.0	43.73 ± 7.2**
Maternal kidney weight (g)	1.799 ± 0.16	1.833 ± 0.15	1.837 ± 0.20	1.906 ± 0.20	1.967 ± 0.19**
Relative maternal kidney weight (% bodyweight)	0.634 ± 0.06	0.631 ± 0.04	0.637 ± 0.07	0.656 ± 0.05	0.698 ± 0.05**
Relative maternal liver weight (% bodyweight)	4.577 ± 0.35	4.829 ± 0.31	4.621 ± 0.28	4.867 ± 0.40***	4.881 ± 0.38**
Fetal bodyweight/litter (g)	5.245 ± 0.26	5.408 ± 0.22	5.217 ± 0.30	4.981 ± 0.31***	4.033 ± 0.40**
No. litters with external malformations/no. examined (%)	0/24 (0)	1/22 (4.5)	0/22 (0)	2/23 (8.7)	8/21** (38.1)
No. litters with soft tissue malformations/no. examined (%)	6/24 (25.0)	9/22 (40.9)	9/22 (40.9)	9/23 (39.1)	17/21** (81.0)
No. litters with skeletal malformations/no. examined (%)	0/24 (0)	0/22 (0)	1/22 (4.5)	10/23** (43.5)	21/21** (100.0)
Skeletal variations				[a]	[a]
NOELs			Fetal	Maternal	

Protocol: CD rats were exposed to ethylene glycol by gavage on gd 6-15. Dams were sacrificed on gd 21 and fetuses from 21-24 litters were evaluated for prenatal developmental toxicity.
Notes:
*=p<0.001, **=p<0.01, ***=p<0.05
[a] See text for description of statistically significant increases in variations.
There were no effects on maternal corrected weight gain, kidney lesions, deaths, abortions, early deliveries, or numbers of implantation sites and corpora lutea.

Maronpot et al. *(79)* treated groups of more than 20 pregnant Fischer 344 rats (100 days old) with ethylene glycol (99.9% pure) in the diet (target doses were 0, 40, 200, and 1,000 mg/kg bw/day) from gd 6-15. Doses corresponded to those of a concurrent two-year assay that demonstrated toxicity at the highest dose. A positive control group received 500 mg/kg hydroxyurea in saline intrapertoneally on gd 11. Pregnant females were sacrificed on gd 21, and the fetuses from each group were randomly allocated to either a visceral and head examination group or a skeletal examination (by maceration and staining) group. Statistical analysis included F test for

continuous data, Fisher's exact test for binomial data, multiple sum of ranks test for non-parametric data, and other paired tests for significant differences (Student's t-test, Cochran's t-test) ($p<0.05$). At least twenty litters or 164-190 fetuses/group were examined. Evaluation of maternal toxicity was limited to clinical signs and bodyweight gain.

The authors stated there were no clinical signs or significant differences between control and treated corrected maternal body weight gains when examined on gd 6, 11, or 21 **[data not shown]**. Fetal data were shown in tables, and no significant effects on fetal length, weight, litter size, or total implantations were reported. The positive controls exhibited numerous major malformations (e.g., tail malformation, twisted limbs, skeletal and heart malformations). These effects were not seen in ethylene glycol-treated groups. A statistically significant ($p<0.001$) increase in incidence of poorly ossified and unossified vertebral centra in fetuses from the 1,000 mg/kg bw/day group (Table 3-5) was described by the study authors as evidence of delayed fetal maturation and suggestive of minimal embryotoxicity. Reduced ossification was not reported as statistically significant in litters. Authors stated that the incidence of major malformations was not increased in treated rats. In the conclusion, the study authors state that the absence of major malformations is interpreted as preliminary indication of lack of teratogenicity of this chemical. **[The Expert Panel selected a fetal NOAEL of 1,000 mg/kg bw/day for this study. Although reduced ossification was noted at the 1,000 mg/kg bw/day dose, the Panel questioned whether a dose that results in reduced ossification but an otherwise normal vertebrae should be identified as a LOAEL. The Panel noted that current thinking on reduced ossification suggests that three or more vertebrae from the same fetus should be affected before the dose producing the effect should be identified as a LOAEL. The data as presented in the paper do not provide that level of detail. However, the lack of other findings (e.g., no change in body weights, no other consistent alterations in skeletal integrity), suggest that the dietary NOAEL is 1000 mg/kg bw/day.]**

Strengths/Weaknesses: The design of this study permits evaluation of dose-response relationships and starts with adequate numbers of animals per group, although the pregnancy rate (~80% in all groups) is surprisingly, but uniformly, low. The dietary mode of administration provides exposure over a longer period of time than gavage dosing. This may more adequately model likely human exposure patterns.

Utility (Adequacy) for CERHR Evaluation Process: This study should be useful for risk extrapolation purposes.

Table 3-5. Prenatal Toxicity Study of Ethylene Glycol in Rats by Maronpot et al. *(79)*.

Effect	Doses (mg/kg bw/day)			
	0	**40**	**200**	**1000**
No. fetuses with poorly ossified vertebrae/no. examined (%)	3/167 (1.8)	1/190 (0.5)	4/164 (2.4)	24/169 (14.2)*
No. fetuses with unossified vertebrae/number examined (%)	19/167 (11.4)	33/190 (17.4)	31/164 (18.9)	44/169 (26.0)*
NOAELs:			Fetal	Maternal

Protocol: Fischer 344 rats were exposed to ethylene glycol in diet on gd 6-15. Dams were sacrificed on gd 21 and fetuses from 20-21 litters/group were evaluated for prenatal developmental toxicity.
Notes:
*=p<0.001, significance obtained when analyzed for fetuses affected but not litters affected.
There were no effects on corrected maternal bodyweight gain or clinical signs, malformations, fetal length or bodyweight, litter size, or total implantations at any dose.
The 500 mg/kg bw hydroxyurea positive control increased visceral and skeletal malformations.

The Expert Panel is aware of a Chinese manuscript by Yin et al. (released around the late 1980's) for a study of ethylene glycol prenatal toxicity study in rats. It is not known if the manuscript was ever published. A literature search revealed that the information was published in an abstract *(80)*. The Expert Panel notes that the majority of results from that study appear to be qualitatively consistent with results from the rat prenatal studies described above.

3.2.1.1.3 Rabbits

Tyl et al. *(68)* studied the prenatal developmental toxicity of ethylene glycol in 5-month-old New Zealand White rabbits in a study conducted according to GLP. Artificially inseminated does, 23-24 per group, received ethylene glycol (98% purity) in deionized/distilled water at doses of 0, 100, 500, 1000, or 2000 mg/kg bw/day by gavage on gd 6-19. Ethylene glycol concentrations in dosing solutions were verified. **[The rationale for dose selection was not presented.]** At scheduled necropsy on gd 30, maternal liver, kidney, and gravid uterus weights were recorded. Ovarian corpora lutea were counted and uterine implantation sites were recorded. Kidneys were examined histologically in 10-17 dams/group. All live fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and variations. Viscera were examined according to the Staples method and the skeleton was stained with alcian blue/alizarin red S. Heads from half the fetuses were fixed in Bouin's solution and examined. The litters were considered the experimental unit. Data were analyzed with the General Linear Trend Models procedures for ANOVA, Bartlett's test for homogeneity of variance, Williams' and Dunnett's multiple comparison tests, and/or Fisher's exact probability test.

At necropsy, 20-22 dams and litters/group were evaluated in the control and three lowest dose group; 9 does and litters were evaluated in the highest dose group due to a high mortality rate. At 2000 mg/kg bw/day, 42% of the does died, three delivered early and one aborted. Kidney weights were slightly increased in the 2000 mg/kg bw/day group (not statistically significant), and necropsy revealed renal toxicity including tubule dilatation and degeneration, epithelial necrosis, and intraluminal oxalate crystal deposition. There were no effects on maternal weight gain or water intake. No statistically significant ($p < 0.05$) effects on pre- or postimplantation loss, number of fetuses, fetal body weight, or sex ratio per litter were observed at any of the doses

tested. There was no evidence of teratogenicity. Findings of this study are summarized in Table 3-6. The study authors identified NOAELs of 1000 mg/kg bw/day for maternal toxicity and at least 2000 mg/kg bw/day for developmental toxicity. **[The Expert Panel concurs with the authors' identification of NOAELs. Very few anomalies were observed among the small number of pups that could be examined from the high dose group. The fact that reduced number of pups at the high dose group resulted from maternal deaths and whole litter loss suggests that pups were not so severely affected that they died.]**

Strengths/Weaknesses: The design of this GLP-compliant study permits evaluation of dose-response relationships and starts with adequate numbers of animals per group, although survival to term among the high dose animals was low.

Utility (Adequacy) for CERHR Evaluation Process: This study should be useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which is unlikely to mirror expected human exposure.

Table 3-6. Prenatal Toxicity Study of Ethylene Glycol in Rabbits by Tyl et al. *(68)*.

Effects	Doses				
	0	**100**	**500**	**1000**	**2000**
Early delivery/no. examined	1/23	1/24	1/24	1/22	3/22
No. maternal deaths/no. examined	0/22	0/23	0/23	0/21	8/19
No. dams with renal lesions /no. examined	4/10	5/10	6/10	5/10	14/17
No. dams with renal crystals /no. examined	0/10	0/10	0/10	0/10	8/17
NOAELs				Maternal	Fetal
Protocol: New Zealand White rabbits were exposed to ethylene glycol by gavage on gd 6-19. Dams were sacrificed on gd 30 and fetuses from 9-22 litters/group were evaluated for prenatal developmental toxicity. Notes: There were no effects on maternal water intake and weight gain, fetal malformations, implantation loss, litter size, fetal body weight, or sex ratio at any dose.					

3.2.1.2 Postnatal Toxicity Studies

Price et al. *(81)* investigated the effects of prenatally administered ethylene glycol on the postnatal development of rats. In a study conducted according to GLP, timed mated Crl:COBS CD (Sprague-Dawley) BR rats, received gavage doses of ethylene glycol (99.6% purity) in distilled water at 0, 250, 1250, 2250 mg/kg bw/day during gd 6-20. The doses were based on findings of previous studies conducted in the laboratory, and concentrations of dose solutions were verified. Thirty-eight to 49 dams/group delivered litters and were sacrificed on pnd 1. Pups from 33-42 litters/group were fostered to untreated control dams on postnatal day 1. The pups were monitored for growth and viability, developmental landmarks, sexual maturation, locomotor activity, and performance on a complex learning task. Pups were intermittently sacrificed to evaluate external and visceral malformations (pnd 1, 4, 22, and 63), skeletal malformations (pnd 22), and liver, kidney and brain histopathology (pnd 4, 22 and 63). Data were analyzed using General Linear Models together with, Bartlett's test for homogeneity of variance, Dunnett's and Williams tests, ANOVA, Chi Square test, and/or Fisher's Exact Probability test.

Incidences of statistically significant findings in the Price et al. *(81*) study are listed in Table 3-7. Maternal body weight gain was reduced in the 2250 mg/kg bw/day group; **[corrected body weights were not reported].** Absolute and relative uterus weight were reduced in the 2250 mg/kg bw/day group. Absolute and relative maternal kidney weight were significantly increased among rats treated with 2250 mg/kg bw/day. **[Note: Tables indicate an increase in absolute and relative kidney weight, but the abstract and discussion state that absolute and relative kidney weights were decreased.]** Histological evaluation revealed treatment-related renal pathology (tubular dilation and regeneration) in dams treated with 2250 or 1250 mg/kg bw/day. Gestational length was significantly prolonged in dams of the 2250 and 1250 mg/kg bw/day groups. Treatment had no effect on the numbers of implantation sites. On pnd 1-4, there were significant increases in pup cumulative mortality/litter and significant reductions in litter size and pup weight gain in the 2250 mg/kg bw/day group. The authors noted that a total of nine pups in the 2250 mg/kg bw/day group (males and females sacrificed on pnd 1, 4, 22, 27, and 63) had hydrocephly versus no pups in the control group. **[The statistical significance of this effect was not discussed.]** In pups sacrificed on pnd 22, there was a significantly increased incidence of skeletal malformations (defects in ribs, sternebrae, and vertebrae) at the 2250 mg/kg bw/day dose level. Significant decreases in kidney weight (absolute in males and relative in females) were observed in the 2250, and 1250 mg/kg bw/day groups on pnd 63; there were no kidney or liver lesions in offspring from the highest dose group. Absolute brain weights were significantly reduced in males and females of the 2250 mg/kg bw/day dose group on pnd 22 and 63. Treatment had no adverse effects on developmental landmarks such as incisor eruption, vaginal opening, testes decent, or wire grasping skills. In addition, no adverse treatment-related effects were noted for exploratory behavior during the preweaning period and performance on a visual discrimination test at 12-14 weeks of age. The study authors concluded that no toxicity was observed at 250 mg/kg bw/day, while maternal and offspring effects were noted at 1250 and 2250 ppm, respectively. **[In agreement with study authors, the Expert Panel concluded that these data indicate a maternal NOAEL of 250 mg/kg bw/day and a developmental toxicity NOAEL of 1250 mg/kg bw/day.]**

Strengths/Weaknesses: This study is GLP-compliant with adequate numbers of animals per group and a design that permits evaluation of dose-response relationships. This study was primarily designed to examine post-natal effects following prenatal exposure, so the design was different than that used by Price et al. *(69*). The highest dose chosen was similar to the middle dose used by Price et al. *(69*) and demonstrated similar effects on neonatal body weight with the caveat that the designs were different.

Utility (Adequacy) for CERHR Evaluation Process: The study is not directly applicable to risk extrapolation. The exposures were delivered as bolus doses by means of gavage administration which is unlikely to mirror expected human exposure. However, it does provide evidence of the transient nature of some of the adverse effects of high dose ethylene glycol exposure during gestation on bodyweight (decreased at pnd 1, 4 and 22 but not different from control at pnd 63) as well as the lack of effect on several developmental landmarks and behavioral tests.

Table 3-7. Postnatal Toxicity Study of Ethylene Glycol in CD Rats by Price et al. *(81)*.

Effect	Doses (mg/kg bw/day)			
	0	**250**	**1250**	**2250**
Maternal bodyweight gain (gd 6-20; g)	106.2	104.6	102.5	84.3**
Gestational length (days; g)	21.26	21.29	21.58**	21.84**
Dams with renal pathology/no. examined	0/18	0/14	4/15	5/15
Maternal kidney weight (g)	2.20	2.24	2.28	2.42***
Maternal relative kidney weight (% bodyweight)	0.79	0.80	0.81	0.87**
Maternal uterine weight (g)	4.62	4.58	4.73	4.08***
Maternal relative uterine weight (% bodyweight)	1.66	1.62	1.68	1.46***
Live litter size, pnd 1 (g)	13.67	12.63	13.58	11.87**
Live litter size, pnd 4 (g)	13.21	12.92	13.26	11.42**
% Cumulative pup mortality/litter - pnd 1	6.7	9.1	9.0	17.1**
% Cumulative pup mortality/litter - pnd 4	9.1	8.4	11.3	22.3**
Pup bodyweight/litter – pnd 1 (g), Male:	6.33	6.47	6.49	5.88**
Female:	5.97	6.07	6.06	5.60**
Pup bodyweight/litter, pnd 22 (g), Male:[a]	42.61	41.96	43.22	38.59***
Female: [a]	41.80	40.56	41.30	37.09***
Pup kidney weight/litter, pnd 22 (g), Male: [a]	0.612	0.616	0.626	0.543***
Female: [a]	0.638	0.630	0.637	0.549**
Pup brain weight/litter, pnd 22 (g), Male: [a]	1.417	1.433	1.409	1.325***
Female: [a]	1.400	1.358	1.320	1.242**
Average pup kidney weight/litter, male – pnd 63 (g) [a]	3.260	3.208	2.981***	2.909***
Relative pup kidney weight/litter, female – pnd 63 (% bodyweight) [a]	1.01	0.96	0.91**	0.92***
No. litters with skeletal malformations/no. examined, pnd 22 (%), Male: [a]	0/23	0/22	0/24	6/20**
Female: [a]	0/24	2/23	0/24	12/20*
NOAELs		Maternal	Fetal	

Protocol: Dams received ethylene glycol by gavage on gd 6-20. Litters were delivered and fostered with untreated dams. Postnatal growth and development were evaluated up to pnd 63 in pups from 33-42 litters/group.

Notes:

[a] Data shown are from only those pups sacrificed on pnd 22 or 63.

*=p<0.001, **=p<0.01, ***=p<0.05

There were no effects on implantation sites, offspring kidney or liver lesions, developmental landmarks, or offspring performance on neurotoxicity testing.

Marr et al. *(82)* investigated the effects of maternal ethylene glycol exposure on pre- and postnatal skeletal development. Pregnant Crl:CD BR VAF/Plus outbred Sprague-Dawley rats were treated with 0 or 2,500 mg/kg bw/day ethylene glycol (99% pure) in water via oral gavage

(5 mL/kg) from gd 6-15. Based on previous studies, a dose sufficient to cause reduced fetal/pup weight was selected. Each dose group was subdivided and 4-7 dams and litters/group were sacrificed on gd 18 or 20 or post-natal day 1, 4, 14, 21, or 63. Endpoints examined were fetal and pup weights and skeletal malformations and degree of ossification (by staining with Alician Blue and Alizarin Red S). Data were analyzed using Student's t-test for normally distributed continuous data and Mann-Whitney U test for comparisons of non-normal continuous data ($p<0.05$).

Results are summarized in Table 3-8. Maternal weight gain during treatment period (gd 6-15) was significantly decreased (by 27%) in the ethylene glycol group, and gestational weight gain was significantly decreased (by 13%) in the treatment group. Fetal body weight was significantly decreased (by ~25%) in the group treated with ethylene glycol and examined on gd 18 and 20. The mean pup body weight per litter was significantly reduced (by 10%) in the treated group examined on post-natal day 1 only; other treated groups examined later had reduced weights that were not significant. The percentages total ossification, sternebrae ossification, and vertebral centra ossification were significantly reduced in the treatment groups on gd 20 and all post-natal days except 63. When these data were covaried with fetal/pup weights, the delayed ossification during gestation was no longer significant, but this effect remained significant for the postnatal data. This suggests that the ossification effects observed postnatally were not influenced by body weight changes, while those seen on gd 20 may have resulted from body weight effects. Percent of malformed pups per litter (predominantly axial skeletal defects) were significantly increased in treated groups examined on all days except post-natal day 63. The authors conclude that offspring effects observed early in development as a result of ethylene glycol treatment may not be permanent since they were not observed at post-natal day 63.

Strengths/Weaknesses: The purpose of this study was to evaluate the potential reversibility of ethylene glycol-related effects seen in previous studies. The strength of the study is that it provides a level of confidence about the long-term impact of the skeletal findings observed in near-term fetuses of high dose litters. Its weaknesses are the absence of historical control data for some of the endpoints observed at the time points used.

Utility (Adequacy) for CERHR Evaluation Process: This study is not directly applicable to risk extrapolation. It does, however, provide evidence that many of the skeletal changes (most of which are variations) appear to be transient.

Table 3-8. Major Effects of Ethylene Glycol in CD Rat Prenatal/Postnatal Study by Marr et al. *(82)*

Effect	Significant Offspring Effects on Each Day of Examination in Treated Versus Control Animals						
	Gd 18	**GD 20**	**Pnd 1**	**Pnd 4**	**Pnd 14**	**Pnd 21**	**Pnd 63**
Pup bodyweight	0.93* vs 1.26	2.75* vs 3.63	6.21* vs 6.90	9.22 vs 10.07	33.20 vs 34.78	51.18 vs 53.28	320.6 vs 338.7
No. malformations per litter/ no. examined (%)	10.3/7 vs 0/7 (76* vs 0)	13.1/7 vs. 0.2/6 (88* vs 1)	7.7/6 vs. 0/6 (95* vs 0)	11.3/4 vs. 1.0/6 (83* vs 6)	5.0/5 vs 0/5 (77* vs 0)	7.0/4 vs 0/7 (87* vs 0)	2.2/5 vs. 0.6/5 (28 vs. 8)
No. litters with skeletal malformations/ no. examined (%)	7/7 vs. 0/7 (100* vs 0)	7/7 vs 1/6 (100* vs 17)	6/6 vs. 0/6 (100* vs 0)	4/4 vs. 3/6 (100 vs 50%)	5/5 vs 0/5 (100* vs 0)	4/4 vs. 0/7 (100* vs 0)	4/5 vs 1/5 (80 vs 20)
Reduced Ossification	[a]	[a]	[a]	[a]	[a]	[a]	[a]

Protocol: Rats gavaged with 0 or 2500 mg/kg bw/day ethylene glycol on gd 6-15. Pups in 4-7 litters/group were examined during late gestation or during the postnatal period.
Notes: *=p<0.05
[a]See text for details.

3.2.2 Inhalation Exposure

3.2.2.1 Mouse

Tyl *(83)* examined mouse prenatal developmental toxicity in a study conducted according to GLP. Crl:CD-1(1CR)BR mice, 25/dose group, were exposed to a respirable aerosol of ethylene glycol (100% purity; mass median aerodynamic diameter of 2.3 μm; whole body exposure) for 6 hours/day at concentrations of 0, 150, 1000, or 2500 mg/m^3 on gd 6-15. Doses were based on results of range finding studies. Chamber concentrations were verified and it was found that chamber concentrations were below target concentrations (119, 888, and 2090 mg/m^3). At scheduled sacrifice on gd 18, 22-25 dams/group were evaluated for body, liver and kidney weight. The gravid uterus was weighed and examined for status of implantation sites. All live fetuses from 22-25 litters were counted, weighed, sexed and examined for external malformations. In one-half of the fetuses, heads were fixed in Bouin's solution and examined and viscera were evaluated by the Staples method. The other half of fetuses were stained with alizarin red S and examined for skeletal malformations. Litters were considered the unit of comparison. Continuous variables were analyzed by the Levene's test, ANOVA, and t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test. Incidence data were evaluated with the Fisher's Exact test. For all statistical tests, a probability value of $P < 0.05$ (two-tailed) was used as the critical level of significance.

Incidences of statistically significant effects in the Tyl *(83)* study are summarized in Table 3-9. Maternal body weight was significantly decreased in the mice exposed to 1000 and 2500 mg/m^3, but corrected weight gain was not affected. The numbers of viable implants per litter were significantly reduced at 2500 mg/m^3. Significant embryo/fetal effects at 1000 and 2500 mg/m^3,

consisted of reduced numbers of live fetuses/litter, reduced fetal body weight/litter, increased numbers of non-viable implants per litter and increased litters with external, visceral, and skeletal malformations and variations. The types of malformations observed included cleft palate, exencephaly, and defects of the nasopharynx, tongue, brain, vertebra, ribs, and face. Authors identified a maternal and developmental toxicity NOEL of 150 mg/m^3. The authors speculated that a majority of the dose received could be from ingestion of ethylene glycol while grooming the fur. Authors estimated that inhalation and ingestion of ethylene glycol resulted in a total dose of 410-606 and 966-1428 mg/kg bw/day in the 1000 and 2500 mg/m^3 groups, respectively. Doses were estimated by measuring ethylene glycol levels in fur to determine potential ingestion and then using inhalation rates with assumed 10% and 90% retention to determine potential inhalation exposure.

Strengths/Weaknesses: This GLP-compliant study was designed to permit the evaluation of exposure-response relationships for whole body inhalation exposures to mice. The author was alert to observe ingestion of test agent from the coats of animals during preening after the animals had been removed from the exposure chambers. Satellite investigations demonstrated that most of the maternal exposure (~94%) was attributed to ingestion of ethylene glycol deposited on the fur during exposure to the aerosol.

Utility (Adequacy) for CERHR Evaluation Process: Tyl *(83)* is a scientifically sound study. The authors identified potential confounding through a second exposure route and then verified and quantified exposure occurring through ingestion. Due to the confounding caused by exposure via two routes, the results of this study are not useful for evaluating effect levels for inhalation of ethylene glycol.

Table 3-9. Major Effects of Ethylene Glycol in Prenatal Toxicity Study in CD-1 Mice by Tyl *(83)*.

Effect	Doses (mg/m^3)			
	0	**150**	**1000[a]**	**2500[b]**
Maternal bodyweight gain (gd 6-15; g)	13.63 ± 1.50	14.33 ± 1.93	11.28 ± 2.46**	9.42 ± 3.06*
Viable implants/litter (g)	10.7 ± 1.8	11.8 ± 2.2	9.3 ± 2.8	8.0 ± 2.9*
Non-viable implants/litter (g)	1.4 ± 1.0	1.1 ± 1.2	2.9 ± 2.0**	4.2 ± 2.9**
% Live fetuses/litter	88.5 ± 8.4	91.3 ± 10.1	76.2 ± 16.5**	65.2 ± 22.9*
Fetal bodyweight/litter (g)	1.33 ± 0.08	1.29 ± 0.10	1.07 ± 0.14*	0.94 ± 0.14*
No. litters with external malformations/ No. examined (%)	1/25 (4.0)	4/22 (18.2)	7/23*** (30.4)	16/22*** (72.7)
No. litters with visceral malformations/ No. examined (%)	2/25 (8.0)	3/22 (13.6)	8/23*** (34.8)	16/22*** (72.7)
No. litters with skeletal malformations/ No. examined (%)	18/25 (72.0)	18/22 (81.8)	23/23*** (100.0)	22/22*** (100.0)
NOELs		Maternal and Fetal		

Protocol: Mice inhaled ethylene glycol mists (whole body exposure) from gd 6-15 and were sacrificed for evaluation of prenatal toxicity in fetuses from 22-25 litters/group on gd 18.
Notes: *=p<0.001, **=p<0.01, ***=p<0.05
[a]Authors estimated a total dose of 410-606 mg/kg bw/day from inhalation and ingestion.
[b]Authors estimated a total dose of 966-1428 mg/kg bw/day from inhalation and ingestion.
There was no effect on corrected maternal weight gain.

Tyl et al. *(84, 85)* next conducted a study to examine the role of ethylene glycol inhalation alone on prenatal developmental toxicity in Crl:CD-1(1CR)BR mice. In a study conducted according to GLP, timed-pregnant CD-1 mice, 30 per dose group, were exposed by nose-only to ethylene glycol (99% purity) aerosol target concentrations of 0, 500, 1000, or 2500 mg/m^3 for 6 hours per day, on gd 6-15. A positive control group received a whole-body exposure to an aerosol target concentration of 2100 mg/m^3. The mass median aerodynamic diameter of the aerosols was 2.6 microns. Two groups of 30 negative control rats were exposed to water vapor. Dose selections were based upon results observed in a previous experiment with whole body inhalation exposure *(83)*. Ethylene glycol concentrations in chambers were measured, and it was found that actual concentrations were below target concentrations **[See table 1 of the study]**. Based on measurement of ethylene glycol levels in the fur of satellite animals, the amounts of ethylene glycol potentially available for oral ingestion were found to be 330 mg/kg bw for nose-only

exposure to 2500 mg/m^3 and 1390 mg/kg bw for whole-body exposure to 2100 mg/m^3. At scheduled sacrifice on gd 18, 22-29 adult mice/treatment group were weighed and kidneys were retained for subsequent microscopic examination. The gravid uterus was weighed and examined and the status of uterine implants was recorded. Each live fetus from a total of 21-29 litters/group was examined for external, visceral, and skeletal malformations. Visceral defects were evaluated using the Staples method and the skeleton was stained with alizarin red S. The heads of half the fetuses were preserved in Bouin's solution and examined. Litters were considered the unit of comparison. Continuous variables were analyzed by the Levene's test, ANOVA, and t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test. Incidence data were evaluated with the Fisher's Exact test. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

Statistically significant effects for the Tyl et al. *(84, 85*) study are listed in Table 3-10. The only maternal effects in the nose only exposure groups were significant increases in absolute kidney weight at 1000 and 2500 mg/m^3 and relative kidney weight at 2500 mg/m^3. There were no kidney lesions or differences in body weight gain, water intake, or the number of total or viable implants/litter. Significant fetal effects in the 2500 mg/m^3 nose-only group included reduced body weights/litter, an increase in one type of skeletal malformation (fused ribs), and increases in some types of skeletal variations. Total malformations and variations were not statistically significant in any of the treatment groups. One type of variation (extra ossification sites in the sagital suture) was increased in all nose-only treatment groups. Significant effects in the whole-body exposure positive control group included increased resorptions, decreased body weights, and increased skeletal malformations and variations. The study authors concluded that the nose-only exposure data indicate a NOEL of 1000 mg/m^3 for developmental toxicity and 500 mg/m^3 for maternal toxicity.

Strengths/Weaknesses: The purpose of this GLP-compliant study was to determine the effect of inhalation of ethylene glycol aerosols under conditions that obviated the confounding observed in Tyl *(83*). The authors recognized that this study also suffered from confounding. The dose received by each animal occurred by two routes because some material was still available for ingestion via preening of the face after removal from the aerosol, although not nearly as much as had been ingested after the whole body exposure. Furthermore, the animals struggled a great deal during restraint, which was required for the nose-only exposure. A subsequent study *(86*) demonstrated that nose-only inhalation exposure of restrained pregnant mice to water aerosol resulted in variations and malformations that were qualitatively similar to those observed in the treated animals of the Tyl *(84*) study, but occurring at a lower incidence. Therefore the Tyl et al. *(86*) study suggests that restraint of the mice could have contributed to the developmental effects observed in the litters of animals inhaling ethylene glycol mists.

Utility (Adequacy) for CERHR Evaluation Process: This study was well conducted. However, due to confounding caused by exposure via two routes and stress associated with restraint of the animals, the results of this study are not useful for evaluating effect levels for inhalation of ethylene glycol.

Table 3-10. Prenatal Toxicity Study of Ethylene Glycol in CD Mice by Tyl *(84)*.

Effect	Concentrations (mg/m^3)			
	0	**500**	**1000**	**2500**
Maternal kidney weight (g)	0.431	0.458	0.466***	0.472**
Maternal relative kidney weight (% bodyweight)	1.354	1.415	1.415	1.444***
Fetal bodyweight/litter (g)	1.289	1.281	1.310	1.184**
No. litters with fused ribs/ No. examined (%)	1/22 (4.5)	2/23 (8.7)	0/26 (0.0)	8/21*** (38.1)
No. litters with extra ossification in sagittal suture/no. examined (%)	12/22 (54.5)	23/23** (100)	24/26** (92.3)	21/21**[a] (100)
NOELs		Maternal	Fetal	

Protocol: Mice inhaled ethylene glycol aerosols (nose only exposure) from gd 6-15 and were sacrificed for evaluation of prenatal toxicity in fetuses from 21-26 litters/group on gd 18.
Notes:
*=p<0.001, **=p<0.01, ***=p<0.05
There were no effects on maternal bodyweight gain, water intake, kidney lesions, implantation sites or significant increases in total external, visceral or skeletal malformations or variations.
[a]There were additional significant individual skeletal variations in litters of the high dose group.
A positive control group exposed to 2100 mg/m^3 ethylene glycol (whole body) had significant increases in resorptions and total skeletal malformations.

3.2.2.2 Rat

Tyl *(83)* examined rat prenatal developmental toxicity in a study conducted according to GLP. Timed-pregnant Crl:COBS CD(SD)BR rats, 25/dose group, were exposed to a respirable aerosol of ethylene glycol (100% purity; mass median aerodynamic diameter of 2.3 μm; whole body exposure) for 6 hours/day at daily doses of 0, 150, 1000, or 2500 mg/m^3 on gd 6-15. Concentrations were based on results of range finding studies. Chamber concentrations were verified and it was found that chamber concentrations were below target concentrations (119, 888, and 2090 mg/m^3). At scheduled sacrifice on gd 21, 20-25 dams/group were evaluated for body liver and kidney weight. The gravid uterus was weighed and examined for status of implantation sites. All live fetuses in 20-24 litters/group were counted, weighed, sexed and examined for external malformations. In one-half of the fetuses, heads were fixed in Bouin's solution and examined and viscera were evaluated by the Staples method. The other half of fetuses were stained with alizarin red S and examined for skeletal malformations. Litters were considered the unit of comparison. Continuous variables were analyzed by the Levene's test, ANOVA, and t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test. Incidence data were evaluated with the Fisher's Exact test. For all statistical tests, a probability value of $P < 0.05$ (two-tailed) was used as the critical level of significance.

Incidences of statistically significant effects in the Tyl *(83)* study are summarized in Table 3-11. Maternal food and water consumption and body weights were unaffected by the chemical exposures. Absolute and relative liver weights of rats in the 2500 mg/m^3 group were increased; organ histopathology was not evaluated. There were no effects on reproductive parameters

including numbers of implantation sites and corpora lutea. Treatment had no effect on prenatal mortality, fetal body weight, or the incidence of external, visceral and skeletal malformations. **[Study tables, but not text, report a statistically significant increase in total visceral malformations for litters of the 2500 mg/m^3 groups but this may be an error since only one fetus was observed to have a visceral malformation.]** In fetuses from the 1000 and 2500 mg/m^3 dose group, some fetal toxicity was expressed as reduced ossification (See table 3-11). **[It does not appear that statistical analyses were conducted for total incidences of skeletal variations within dose groups.]** Authors identified a fetal NOEL of 150 mg/m^3. **[The Expert Panel disagreed with the authors selection of a NOEL. Reduced ossifications, deemed compound related by authors, were seen at different sites in animals of the 1000 and 2500 mg/m^3 exposure groups. The lack of a dose response relationship at ≥1000 mg/m^3 suggests that the NOAEL exceeds 150 mg/m^3.]** The role of inhaled ethylene glycol in causing effects in this study was uncertain because the authors speculated that a majority of the dose received could have been from ingestion of ethylene glycol while grooming the fur. Authors estimated that inhalation and ingestion of ethylene glycol resulted in a total dose of 279-402 and 656-947 mg/kg bw/day in the 1000 and 2500 mg/m^3 groups, respectively. The dose estimates were formulated by measuring ethylene glycol levels in fur to determine potential ingestion and then using inhalation rates with assumed 10% and 90% retention to determine potential inhalation exposure.

Strengths/Weaknesses: This GLP-compliant study was designed to permit the evaluation of exposure-response relationships for whole body inhalation exposures to rats. As in the case with the study in mice, the exposed animals ingested test agent while preening after they had been removed from the exposure chambers. Confounding resulted from exposure occurring through two exposure routes.

Utility (Adequacy) for CERHR Evaluation Process: Due to the confounding caused by exposure via two routes, the results of this study are not useful for evaluating effect levels for inhalation of ethylene glycol.

Table 3-11. Prenatal Toxicity Study in CD Rats by Tyl *(83)*.

Effect	Doses (mg/m^3)			
	0	**150**	**1000[a]**	**2500[b]**
Maternal liver weight (g)	13.84 ± 1.72	14.01 ± 1.10	14.30 ± 1.39	15.00 ± 1.31***
Maternal Relative liver weight (% bodyweight)	4.76 ± 0.34	4.88 ± 0.34	4.86 ± 0.32	5.07 ± 0.34***
No. litters with some poorly ossified proximal phalanges/no. examined (%)	14/22 (63.6)	18/22 (81.8)	22/24*** (91.7)	17/20 (85.0)
No. litters with some poorly ossified metatarsals/no. examined (%)	18/22 (81.8)	20/22 (90.9)	24/24*** (100.0)	18/20 (90.0)
No. litters with poorly ossified humerus/no. examined (%)	0/22 (0.0)	0/22 (0.0)	1/24 (4.2)	4/20*** (20.0)
No. litters with poorly ossified zygomatic arch/no. examined (%)	9/22 (40.9)	8/22 (36.4)	10/24 (41.7)	15/20*** (75.0)
NOELs		Fetal		

Protocol: Rats inhaled ethylene glycol mists (whole body exposure) from gd 6-15 and were sacrificed for evaluation of prenatal toxicity in fetuses from 20-24 litters/group on gd 21.

Notes:

*=p<0.001, **=p<0.01, ***=p<0.05

[a]Authors estimated a total dose of 279-402 mg/kg bw/day from inhalation and ingestion.

[b] Authors estimated a total dose of 656-947 mg/kg bw/day from inhalation and ingestion.

There were no effects on maternal bodyweight, food or water intake, or implantation sites, prenatal mortality, fetal body weight, or total external, visceral, or skeletal malformations.

3.2.3 Dermal Exposure Studies

Tyl *(74)* studied prenatal development toxicity resulting from dermal exposure to ethylene glycol in mice in order to determine if skin absorption contributed to dermal toxicity in a previous mouse whole body inhalation exposure experiment *(83)*. The study was conducted according to GLP. Timed-pregnant Crl:CD-1 (ICR) BR mice (30/dose group) were exposed to ethylene glycol (100% purity) by occluded cutaneous application for 6 hours per day on gd 6-15 at doses of 0, 12.5, 50, or 100% ethylene glycol (w/v). Deionized water was used as the vehicle for the two lower doses. According to the study authors, those doses are equivalent to 0, 404, 1677 and 3549 mg/kg bw. A positive control group of 30 mice received a gavage dose of ethylene glycol in deionized water at 3000 mg/kg bw/day on gd 6-15. The rationale for the dermal doses was not stated; but the oral dose was selected because it produced developmental toxicity in a study by Price et al. *(69)*. Concentrations of dosing solutions were verified. At scheduled sacrifice on gd 18, renal histopathology was examined in dams of the high dose and control groups and implantation sites were examined in all dams (18-29 dams/group). A total of 16-23 litters/group were examined. In all fetuses, viscera were examined by the Staples method and skeletal effects were examined by staining with alizarin red S. Heads from half the fetuses were fixed in Bouin's

solution and examined. The litter was the unit of comparison for analyses of data. Continuous variables were analyzed by Levene's test for equal variances, ANOVA, and t-tests with Bonferroni probabilities. Nonparametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test. Fisher's Exact Test was used to evaluate incidence data. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

Statistically significant results of the Tyl *(74)* study are outlined in Table 3-12. Dermal-treated dams experienced no chemical-related clinical signs or effects on body weight, water intake, liver or kidney weight, or renal histopathology. The number of resorptions or total implants/litter were not affected in the dermally treated group. The only fetal effect observed with dermal treatment was a statistically significant increase in the incidence of two skeletal variations (reduced ossification of skull bone and phalanges) in the 3549 mg/kg bw group. Total external, visceral, or skeletal malformations were not significantly increased in any dose group. Fetuses in the positive control group experienced reduced body weights and increases in visceral malformations and skeletal malformations and variations. The authors concluded that the maternal and developmental toxicity NOEL for dermal exposure to ethylene glycol was at or near 3549 mg/kg bw/day for undiluted (100%) ethylene glycol.

Strengths/Weaknesses: This GLP-compliant study was designed to assess the potential developmental toxicity of cutaneously applied ethylene glycol on pregnant mice. The occlusion of the exposure sites precluded possible ingestion of ethylene glycol and thereby minimized potential confounding by multiple routes of exposure.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful because it establishes the lack of effect (or very low impact) on development of dermally applied ethylene glycol in mice. Because blood levels were not measured, it is not known how much, if any, of the ethylene glycol was absorbed. This study is not useful for extrapolation of doses to humans.

Table 3-12. Major Effects Associated with Dermally Applied Ethylene Glycol in Prenatal Toxicity Study in CD-1 Mice by Tyl *(74)*.

Effect	Doses (mg/kg bw/day)			
	0	**404**	**1677**	**3549**
Maternal corrected bodyweight change(g)	3.310	4.040	4.114	5.044***
No. litters with poorly ossified parietal/no. examined (%)	7/23 (30.4)	8/20 (40.0)	5/17 (29.4)	12/18*** (66.7)
No. litters with unossified phalanges/no. examined (%)	8/23 (34.8)	12/20 (60.0)	8/17 (47.1)	13/18*** (72.2)
NOELs				Maternal and Fetal
Protocol: Mice were treated dermally with 0, 12.5, 50, or 100% ethylene glycol for 6 hours/day on gd 6-15. Sacrifice occurred on gd 18 for evaluation of prenatal developmental toxicity in fetuses from 17-23 litters/group. **Notes:** *=p<0.001, **=p<0.01, ***=p<0.05 There were no effects on maternal bodyweight, water intake, liver or kidney weight or renal histopathology or total visceral, skeletal, or external malformations or variations. A positive control group treated with ethylene glycol by gavage had increases in visceral and skeletal malformations.				

3.2.4 Mechanistic Studies

A series of studies were performed to determine if ethylene glycol-induced developmental toxicity is caused by ethylene glycol, its metabolites such as glycolic acid, or from metabolic acidosis, or hyperosmolarity. In order to provide perspective, Table 3-13 outlines blood ethylene glycol and glycolic acid levels associated with developmental toxicity in rats and human poisonings. Additional studies have examined the developmental toxicity of other ethylene glycol metabolites, but there are no reports of those metabolites being detected in blood at significant levels in animal studies. For example, the blood level of oxalic acid was usually below the quantifiable limit of 4.9 µg/g **[0.054 mM]** in rats dosed with up to 2,500 mg/kg bw ethylene glycol *(33)*. In rats gavaged with 1000 mg/kg bw ethylene glycol, blood levels of unresolved glyoxylate/glyoxal were about 2 orders of magnitude lower than ethylene glycol blood levels; glycolaldehyde was only detected once during a 12-hour period following dosing at a level roughly 2 orders of magnitude lower than ethylene glycol *(30, 32)*. The detection limit for glycolaldehyde was 33 ng/100 µL **[0.006 mM]**.

Table 3-13. Examples of Ethylene Glycol and Glycolic Acid Blood Levels.

Ethylene Glycol Exposure (Reference)	Estimated Peak Blood Ethylene Glycol Level (Reference)	Estimated Peak Blood Glycolic acid Level (Reference)
1,000 mg/kg bw/day; LOEL for developmental toxicity in rats *(77)*	14.3-21 mM *(30, 32, 33)*	4.8 mM *(33)*
2,500 mg/kg bw/day; teratogenic concentration in rats *(45)*	45-57 mM *(33)*	5.7-8.8 mM *(45, 33)*
Human poisoning cases *(39, 87)*	1-130 mM* *(39, 87)*	0-30 mM* *(39, 87)*

*May not be peak concentrations since they were measured from 1.5-30 hours following exposure.

In vivo and *in vitro* mechanistic studies are presented below in chronological order, according to the dates they were published.

Grafton and Hansen *(88)* investigated the direct effects of ethylene glycol on the *in vitro* development of whole CD rat embryos. Gd 10.5 embryos (11-18/group) were cultured in rat serum-containing medium with 0, 30, or 40 µL/mL **[0, 535 or 714 mM]** ethylene glycol (99.6% pure). The embryos were cultured for 8 hours (either hours 0-8 or hours 8-16 of a 48-hour culture period). Exposure duration was based on preliminary tests demonstrating that exposure to 30 µL/mL for 16 hours produced 100% embryo lethality. **[There was no discussion on rationale for dose selection]**. Following exposure, embryos were washed and transferred to fresh serum for either 40 or 32 hours. Embryos were evaluated for development, viability, and anomalies. Data were analyzed using one-tailed Dunnett's test for continuous data and Chi-square for comparisons of discrete data ($p<0.05$). One embryo in each exposure group was dead after the culture period. Significant effects in the 40 µL/mL group included decreased crown-rump length and DNA content at hours 0-8 and 8-16 and decreased head length and somite numbers at hours 0-8. Significantly decreased protein content was seen in embryos exposed to both dose levels of ethylene glycol with dose-related increases in hypoplastic telencephalon, lack of optic and otic development, absent hindlimb bud and absent yolk sac circulation also observed. Most of the lesions were more numerous in the groups exposed during hours 8-16 (Table 3-14). Since the enzymes involved in ethylene glycol metabolism, alcohol and aldehyde dehydrogenases, were not present in the culture medium, the authors speculate that the observed effects were due to ethylene glycol itself.

Strengths/Weaknesses: The whole embryo culture technique is a good choice to examine the direct embryotoxic potential of ethylene glycol due to the embryo's very limited ability to metabolize the compound. Adequate numbers of embryos were evaluated in each group, and statistical analysis appears appropriate. However, no rationale was presented for dose selection or for time of exposure. The levels of ethylene glycol that were used are much higher than blood levels observed with teratogenic exposures, and the levels producing abnormalities *in vitro* would probably never be achieved *in vivo*.

Utility (Adequacy) for CERHR Evaluation Process: Because of the high concentrations of ethylene glycol used, this work is of no utility for the CERHR evaluation process.

Table 3-14. Embryotoxicity Observed Following *In Vitro* Exposure to Ethylene Glycol, Grafton and Hansen *(88)*.

Concentration (µL/mL)	Exposure Period (hr)	Number of Embryos With Defect/ Number of Embryos Evaluated				
		Yolk Sac Circulation Absent	Hypoplastic Telencephalon	Absent Hindlimb Bud	No Otic Development	No Optic Development
0	-	0/18	0/18	0/18	0/18	0/18
30	0-8	2/12	1/12	1/12	1/12	1/12
30	8-16	7/15	4/15	5/15	0/15	0/15
40	0-8	4/16	0/16	2/16	5/16	5/16
40	8-16	11/15	2/15	9/15	2/15	7/15

In a two-phase study, Khera *(89)* investigated the roles of maternal acid-base electrolyte imbalance and histological changes in maternal/extraembryonic tissues in ethylene glycol-induced developmental toxicity. Pregnant Crl:Sprague-Dawley rats were treated on gd 11 (plug day = gd 1) with ethylene glycol (water vehicle) using several different exposure routes and doses, depending on the type of endpoint to be examined. The ethylene glycol was of unspecified purity but was described as analytical grade. Data were analyzed using Student's t-test ($p<0.05$).

An acid-base electrolyte imbalance study was first conducted and it was demonstrated that rats experience increased osmolal gap, hyperosmolality, and metabolic acidosis following exposure to 1,250, 2,500 or 5,000 mg/kg bw ethylene glycol by gavage or 3,333 mg/kg bw ethylene glycol administered subcutaneously. Maternal toxicity (depressed reflexes, ataxia, lethargy) was seen in rats dosed orally with 5,000 mg/kg ethylene glycol or subcutaneously with 3,333 mg/kg ethylene glycol; these symptoms were less marked at the other doses. Combined ethylene glycol-sodium bicarbonate treatment resulted in significantly, but not entirely, reduced osmolal gap, acidosis, and osmolality.

In the teratology portion of the experiment, Khera *(89)* dosed 10-15 dams/group with 2,800 or 3,333 mg/kg bw ethylene glycol by s.c. injection on gd 11. In parallel with each ethylene glycol treatment scenario, animals were simultaneously given 530 mg/kg sodium bicarbonate by gavage and drinking water containing 2.65 mg/mL sodium bicarbonate ($NaHCO_3$). This parallel group allowed the researchers to observe any effects sodium bicarbonate treatment might have on acid-base-electrolyte imbalance or fetal anomalies associated with ethylene glycol exposure. Negative water control groups were also included in the experimental design. Three of 13 dams in the 3,333 mg/kg group died; no maternal deaths were seen when sodium bicarbonate was simultaneously administered. Two-thirds of the fetuses were examined for skeletal effects and the remaining fetuses were checked for visceral defects. Fetal body weights were reduced in the group treated with 3,333 mg/kg bw ethylene glycol and skeletal anomalies (ribs, vertebrae, and sternebrae) were increased in the 2,800 and 3,333 mg/kg bw ethylene glycol groups. As noted in Table 3-15, treatment with sodium bicarbonate mitigated the fetal body weight effect in the 3,333 mg/kg group, and significantly reduced incidences of total skeletal anomalies in the 2,800 and 3,333 mg/kg groups. **[Reporting of statistical significance was limited to comparisons**

between groups dosed with ethylene glycol and groups dosed with ethylene glycol in addition to sodium bicarbonate.]

Strengths/Weaknesses: Generally adequate numbers of animals were used in each experimental group, and fetuses were evaluated for signs of developmental toxicity using appropriate methods. This study monitored various clinical chemistry parameters following a teratogenic dose of ethylene glycol (*e.g.*, plasma pH, PCO_2, bicarbonate levels, electrolyte levels, hemoglobin, osmolality). Sodium bicarbonate was administered to reverse some of the physiological effects of ethylene glycol; teratogenicity as well as the clinical chemistry parameters were determined in animals with/without sodium bicarbonate. No rationale was presented for the doses or the treatment time selected. Malformation data were not presented on a per litter basis. Insufficient detail was presented to determine if the statistical analysis was appropriate.

Utility (Adequacy) for CERHR Evaluation Process: These data would appear to be of little use for the CERHR evaluation process. The authors demonstrated that some of the teratogenic effects of the high dose of ethylene glycol could be due to the metabolic acidosis produced by the chemical.

Table 3-15. Fetal Effects of Ethylene Glycol Exposure and Sodium Bicarbonate Treatment in Rats, Khera *(89)*.

Treatment	Parameter	
	Fetal Weight (g)	**No. Fetuses With Skeletal Anomalies/No. examined [%]**
Water Control	5.2	4/110 [3.6]
$NaHCO_3$ Control	5.2	11/106 [10.4]
2,800 mg/kg bw Ethylene Glycol	4.8	55/136 [40.4]
2,800 mg/kg bw Ethylene Glycol + $NaHCO_3$	5.1	20/128 [15.6]*
3,333 mg/kg bw Ethylene Glycol	4.6	70/82 [85.4]
3,333 mg/kg bw Ethylene Glycol + $NaHCO_3$	4.9*	46/83 [55.4]*

*Statistically significantly difference ($p<0.05$) compared to group dosed with the same concentration of ethylene glycol but no $NaHCO_3$.

A histopathological examination of maternal and placental tissues was performed by Khera *(89)* on dams treated with ethylene glycol on gd 7-13 with 3,333 mg/kg bw subcutaneously, or with 500 mg/kg bw by gavage **[this group is apparently mistakenly described as "5,000 mg/kg/day" in the Methods section, but is later described as "500 mg/kg/day" in the Results section and in Figure 8]**. Seven of 8 conceptuses examined at 24 hours post-dosing in the 3,333 mg/kg ethylene glycol group exhibited lesions in the chorioallantoic labyrinth and/or allantois, while none of these lesions were observed in controls. Simultaneous administration of sodium bicarbonate reduced this number to 4/12. A significant increase in the ratio of maternal to fetal vascular area in the labyrinth were seen at 48 hours post-dosing in the 3,333 mg/kg bw ethylene glycol group; the ratio was reduced, but still significantly increased compared to control, when sodium bicarbonate was simultaneously administered. Larger maternal vascular spaces and proportionally smaller allantoic villi and placental basal zone were also seen in the 500 mg/kg/day oral gavage dose group.

Khera *(89)* postulated that maternal metabolic acidosis and hyperosmolality may have contributed to reduction in villigenesis and developmental effects. The ethylene glycol-induced homeostatic changes in the mother and histologic changes in placentae were postulated to affect embryonic nutrition and, consequently, development.

Strengths/Weaknesses: This study used a dose of ethylene glycol that had previously been shown to be developmentally toxic. Although the uteri of 3-8 dams/test group were fixed, it is not clear how many conceptuses from each litter were examined.

Utility (Adequacy) for CERHR Evaluation Process: Although they are interesting, the results from this study appear to be of little use for the CERHR evaluation process. The results are consistent with the author's hypothesis that the observed placental changes could have affected embryonic nutrition.

Carney et al. *(90)* conducted two *in vitro* studies in rat embryos in order to determine the role of ethylene glycol, glycolic acid, acidity, and hyperosmolarity in ethylene glycol-induced developmental toxicity. In each study conducted according to GLP, embryos were obtained from Crl:CD (Sprague-Dawley) rats on gd 10.5 and treated with ethylene glycol, glycolic acid, or sodium glycolate (all chemicals greater than 98% pure) for 46 hours. GD 10.5 embryos were selected to maintain consistency with the studies conducted by Grafton and Hansen *(88)* and Khera *(89)*. Following the exposure period, embryos were monitored for viability, growth, and morphology. Before and after the exposure period, concentrations of ethylene glycol and glycolic acid were monitored by GC/MS to verify target concentrations and to ensure that ethylene glycol was not metabolized to glycolic acid by embryos. The pH of the media was also monitored. Statistical analyses included the Fisher's exact test for percentage data and Bartlett's test, ANOVA, Dunnett's test, and/or the Wilcoxon Rank Sum test with Bonferroni's correction for continuous data.

In the first study, Carney et al. *(90)* exposed 10 embryos/group to 0.5, 2.5, 12.5, 25.0, or 50.0 mM ethylene glycol or glycolic acid for 46 hours. A positive control group of 10 embryos was exposed to 1.0 mM sodium valproate. **[A negative control group was also used, but treatment of that group was not specified.]** The only effect in embryos treated with ethylene glycol was a very slight but significant reduction in the Brown-Fabro morphology score *(91)* in the 50 mM group which the authors interpreted as an insignificant delay in development. In contrast, numerous developmental effects were noted in the embryos treated with ≥12.5 mM glycolic acid including significant reductions in crown-rump length, somite number, morphology score, and embryo protein content. Embryo viability and yolk sac protein content were significantly reduced with exposure to ≥25 mM glycolic acid. Dysmorphogenesis was noted in seventy and sixty percent of embryos in the 12.5 and 50 mM glycolic acid groups, respectively, while no dysmorphogenesis was noted in controls. The structures in which morphological abnormalities were most commonly observed included the maxillary process, mid-facial regions, and telencephalic hemispheres. In the positive control valproate group, 100% dysmorphogenesis was observed in addition to signs of decreased growth.

In the second study, Carney et al. *(90)* incubated 12 embryos/group in control media with a pH of 7.41, control media with a pH of 6.74, media with 12.5 mM glycolic acid (pH=6.74), or media with 12.5 mM sodium glycolate (pH=7.42). Results of that study are listed in Table 3-16. Adverse effects on growth and dysmorphogenesis in the 12.5 mM glycolic acid group were consistent to those observed in the 12.5 mM glycolic acid group in the first study. Effects in the 12.5 mM sodium glycolate group were virtually identical, but the magnitude of effects was

slightly less. For example the percentage of dysmorphogenic embryos in the glycolic acid and sodium glycolate groups was 67% and 58%, respectively. Significant effects noted in the pH 6.74 control group were reductions in head length and embryo and yolk sac protein content.

Based on the results of these experiments, Carney et al. *(90*) concluded that glycolic acid is the proximate developmental toxicant following ethylene glycol exposure and that acidity of culture media is only a minor contributor to the effects observed *in vitro*. The authors further explain that acidification of culture media does not simulate metabolic acidosis occurring *in vivo* since it is a dynamic process that involves other factors such as reductions in PCO_2 and bicarbonate along with increases in lactate and glucose. Lastly, the authors noted that development was not apparently affected by the osmolarity of culture media since no major effects were noted with exposure to a very hyperosmolar solution of 50 mM ethylene glycol.

Strengths/Weaknesses: The whole embryo culture technique is a good choice to examine the direct embryotoxic potential of ethylene glycol and glycolic acid due to the embryo's very limited ability to metabolize these compounds. The concentrations of both compounds were determined at the beginning as well as at the end of the culture period to insure the correct starting concentration as well as to determine if there was embryonic metabolism. The doses of chemicals used were chosen based on previous pharmacokinetic data. The study was also conducted under GLP conditions, and a positive control group was added to the study. Additionally, the types of dysmorphogenesis observed are similar to malformations produced *in vivo*. In the second experiment, a group was added to control for decreased pH as well as adding a group to specifically test the effect of the glycolate anion. The number of embryos treated in each group in both studies is somewhat small (N = 5-12 embryos) and the developmental stage at the beginning of the culture period was not controlled (although not specifically stated, the animals were shipped from the supplier after verification of pregnancy and this probably resulted from an overnight breed).

Utility (Adequacy) for CERHR Evaluation Process: These data are of high utility for defining the proximate developmental toxicant following ethylene glycol exposure in rats. This is a well-done study which demonstrated developmental toxicity of glycolic acid specifically that was not due to changes in pH or hyperosmotic conditions in the media. This study also demonstrated no developmental toxicity of ethylene glycol at concentrations similar to those observed in *in vivo* studies.

Table 3-16. Effects Observed in *In Vitro* Study of Ethylene Glycol by Carney et al. *(90)*.

Effect	Treatment			
	Control (pH=7.41)	**Control (pH=6.74)**	**12.5 mM Glycolic Acid (pH=6.74)**	**12.5 mM Sodium Glycolate (pH=7.42)**
Crown-rump length (mm)	4.8	4.5	3.6*	43.*
Head length (mm)	2.6	2.3*	2.1*	2.2*
Embryo protein [units not specified]	989	771*	399*	596*
No. somites	31.8	30.5	20.2*	25.8*
Morphology score	48.9	47.9	41.0*	43.9*
Visceral yolk sac (mm)	6.9	6.6	5.8*	6.1*
Visceral yolk sac protein (μg)	456	345*	301*	330*
No. dysmorphogenic/ no. evaluated (%)	0/12 (0)	1/12 (8%)	8/12 (67%)*	7/12 (58%)*
Notes: *=p<0.05				

In a subsequent publication, Carney et al. *(45)* examined the roles of glycolic acid and metabolic acidosis in producing developmental toxicity *in vivo* using Crl:Sprague-Dawley rats in a study conducted according to GLP. Twenty-five rats/group received one of the following treatments on gd 6-15: 1) gavage with 2,500 mg/kg bw ethylene glycol (40.3 mmol/kg bw; 99.98% purity); 2) gavage with 650 mg/kg bw glycolic acid (8.5 mmol/kg bw; 99.7% purity); and 3) subcutaneous injection with 833 mg/kg bw sodium glycolate (8.5 mmol/kg bw; ≥98% purity), or 4) gavage with the deionized water vehicle. Concentrations of dosing solutions were verified. In the first phase of the study, it was verified that each treatment produced identical peak serum glycolate levels (8.4-8.8 mM) and that metabolic acidosis was produced in the groups receiving ethylene glycol or glycolic acid by gavage but not in the group receiving sodium glycolate subcutaneously. However, the AUC for glycolate was found to be three fold higher when ethylene glycol exposure data were compared to glycolic acid or sodium glycolate exposure data. Following sacrifice of dams on gd 21, at least half the fetuses were dissected under a stereomicroscope and examined for visceral malformations according to the Staples method; the heads of those fetuses were preserved in Bouin's solution and examined. The skeletons of the remaining fetuses were evaluated by staining with alizarin red-S. Data were evaluated by Bartlett's test, ANOVA, 2-sided Dunnett's test, Wilcoxon Rank-Sum test with Bonferroni's corrections, and/or Fisher exact probability test.

A total of 21-25 litters/group were examined. Treatment related deaths were observed in 4 dams of the glycolic acid group. Resorptions were slightly elevated in all treatment groups and reached statistical significance in the ethylene glycol group. Fetal weights were significantly reduced in all 3 treatment groups with the effect most pronounced with ethylene glycol treatment. The

primary effects in the ethylene glycol group included significantly increased incidences of axial skeleton defects, cranial neural tube defects, craniofacial defects, abdominal wall defects, and skeletal variations. The pattern of malformations in the glycolic acid group was similar to that of the ethylene glycol group except that there were no cranial neural tube and craniofacial defects observed. Incidences of malformations in each treatment group are outlined in Table 3-17. Several skeletal malformations were significantly increased in the glycolic acid group but occurred at a lower incidence than the ethylene glycol group. The only significant effects in the sodium glycolate group were increased incidence of skeletal variations that were also observed with ethylene glycol treatment. The severity of malformations in the sodium glycolate group was less than that of the ethylene glycol and glycolic acid groups. All malformations seen in the glycolic acid and sodium glycolate group also occurred in the ethylene glycol group. According to the study authors, the data indicate that glycolate ion alone can produce developmental toxicity but that metabolic acidosis is a major exacerbating factor. The authors also speculated that the reason why cranial neural tube and craniofacial defects were observed only with exposure to ethylene glycol was because of the 3-fold higher glycolate AUC that occurred with ethylene glycol versus glycolic acid or sodium glycolate treatment.

Strengths/Weaknesses: This study is a well-conducted, GLP study performed in accordance with regulatory guidelines and standard practices using appropriate numbers of animals and statistical analyses. Fetuses were evaluated for signs of developmental toxicity using appropriate methods. Although only single doses of each compound were used, pharmacokinetic analyses were conducted on gd 10 to insure that plasma levels of glycolic acid were similar in the treatment groups. The defects observed in this study after ethylene glycol treatment are similar to those observed by Price et al. *(69*).

Utility (Adequacy) for CERHR Evaluation Process: This study provides data suggesting that both glycolic acid as well as the metabolic acidosis produced by ethylene glycol or glycolic acid are involved in the mechanism of teratogenicity. There was some evidence of maternal toxicity in the groups administered ethylene glycol and glycolic acid, and this may have contributed to the observed developmental toxicity. There was no evidence of maternal toxicity in the sodium glycolate group (except for increased liver weight), and no malformations (only skeletal variations) were observed in this group. The increased AUC for glycolic acid after ethylene glycol administration (which would probably be further exacerbated by repeated dosing with ethylene glycol [not discussed by the authors]), provides a plausible explanation for the higher incidences and more severe defects produced by ethylene glycol.

Table 3-17. Incidence of Rat Malformations in Carney et al. *(45)* study.

Malformation	% of Affected Litters in EachTreatment Group			
	Control	Glycolic acid	Sodium Glycolate	Ethylene Glycol
Meningoencephalocele	4.2	0	0	25.0*
Exencephaly	0	0	0	25.0*
Cleft lip	0	0	0	29.2*
Cleft palate	0	0	0	29.2*
Omphalocele	0	0	0	54.2*
Dilated cerebral ventricles	0	19.0	0	33.3*
Hemivertebrae	0	71.4*	4.0	95.8*
Extra vertebrae	0	4.8	0	29.2*
Missing vertebrae	0	28.6*	0	62.5*
Fused vertebrae	0	19.0	0	75.0*
Fused centra	0	4.8	0	33.3*
Fused ribs	0	42.9*	0	95.8*
Missing ribs	0	71.4*	4.0	91.7*

*Significantly different from control values (α=0.05).

Munley et al. *(92)* examined the developmental toxicity of glycolic acid in Crl:CD®BR rats. Twenty-five dams/group were randomly assigned to groups and dosed with 0 (water control), 75, 150, 300, or 600 mg/kg bw glycolic acid (99.6% purity) in water by gavage on gd 7-21. Dose levels were based on a screening study that demonstrated maternal and developmental toxicity at 350 mg/kg bw and greater. **[Blood levels of glycolic acid were not measured. However, based on data by Carney et al., *(45)* it is expected that the glycolic acid blood level in the 600 mg/kg bw group would be 8 mM or lower; that glycolic acid blood level was obtained following gavage treatment with 2,500 mg/kg bw ethylene glycol *(33, 45)*.]** Concentrations of dosing solutions were verified through an acid/base titration method. Maternal toxicity was evaluated by assessing body weight and food intake. On gd 22, dams were euthanized for an evaluation of implantation sites and fetal toxicity. Uteri of apparently non-pregnant dams were stained with ammonium sulfide to check for resorptions. A total of 23-25 litters/group were evaluated. Fetuses were sexed, weighed, and examined for external malformations. Skeletal effects were examined in all fetuses by fixing them in 70% ethanol, macerating with 1% potassium hydroxide, and staining with alizarin red S. Visceral effects were examined according to the Staples method in every other fetus and in fetuses with skeletal malformations. Heads of half the fetuses were fixed in Bouin's solution and examined. Statistical analyses for maternal effects included ANOVA or the Cochran-Armitage test. The litter was considered the statistical unit for evaluation of developmental effects and statistical analyses included Jonckheere's test and analysis of covariance (ANCOVA).

Maternal body weight gain during treatment and final bodyweight adjusted for gravid uterine weight were significantly reduced in the 600 mg/kg bw/day group. A slight but significant reduction in food intake was noted at this dose only from gd 21 to 22. Clinical signs observed in

dams of the 600 mg/kg bw/day group included abnormal gait, lung noises (wheezing and/or rattling), and irregular respiration. Lung noises (wheezing and/or rattling) were also heard in 2 dams of the 300 mg/kg bw/day group. There were no effects on reproductive parameters including resorptions, numbers of corpora lutea and implantation sites, litter size, or sex ratio at any dose. Statistically significant effects in fetuses are outlined in Table 3-18. Developmental toxicity was observed in the 600 mg/kg bw/day group and included significantly reduced fetal weight and increased numbers of litters containing fetuses with skeletal malformations and variations. Malformations included missing ribs and fused ribs, vertebra, and sternebra. In the 300 mg/kg bw/day dose group, fused ribs and sternebra were noted in 2 fetuses from 2 litters (p=0.055); the authors considered the effect to be relevant to treatment since it was consistent with effects noted at 600 mg/kg bw/day. Fetal mortality was not affected at any dose. The study authors identified 150 mg/kg bw/day as a maternal and developmental NOEL.

Strengths/Weaknesses: This is a well-conducted study done according to regulatory guidelines and standard practices using appropriate numbers of animals. The length of the dosing period was slightly different than that used by Carney et al. *(45*). Plasma glycolic acid levels were not determined in this study, but the high dose of glycolic acid administered was nearly the same as that administered by Carney et al. *(45*) and should have produced similar blood levels. Although many of the skeletal malformations observed in this study are the same as those observed by Carney et al., *(45*) the visceral and external defects observed by Carney were not observed in the current study. Malformations were only observed at the highest dose used, and that dose produced maternal toxicity (decreased body weight, weight gain and increased clinical signs). Some of the clinical signs observed in this study were similar to those observed by Carney et al. *(45*).

Utility (Adequacy) for CERHR Evaluation Process: These data are useful for the CERHR evaluation process. Data from this study suggests that glycolic acid may be teratogenic, but only at doses that also produce maternal toxicity.

Table 3-18. Incidence of Skeletal Malformations in Rats Administered Glycolic Acid by Munley et al. *(92*).

Malformation	No. Affected Litters/No. Examined (%) in Control and Two Highest Dose Groups		
	Control	300 mg/kg bw	600 mg/kg bw
Missing rib	0	0	3/23 (13.0)*
Fused ribs	0	2/23 (8.7)**	9/23 (39.1)*
Fused sternebra	0	0	3/23 (13.0)*
Non-fused sternebra	4.0	1/23 (4.3)	5/23 (21.7)*
Fused vertebra	0	2/23 (8.7)**	6/23 (26.1)*
Hemivertebra	0	1/23 (4.3)	8/23 (34.8)*

*Statistically significant (p≤0.05)
**p=0.0555

Klug et al. *(93*) conducted an *in vitro* study to examine the developmental toxicity of ethylene glycol and its metabolites in rat embryos. Embryos were obtained from Wistar rats (Bor:

Wisw/SPF, TNO) on gd 9.5 and incubated for 48 hours in media containing 50-200 mM ethylene glycol, 0.03-0.3 mM glycolaldehyde, 1-10 mM glycolic acid, 3-6 mM glyoxal, 0.3-1 mM glyoxylic acid, or 1-2 mM oxalic acid **[purity of chemicals not specified]**. Untreated control embryos were also examined. A total of 38 embryos in control groups and 5-19 embryos/treatment group were evaluated for viability, growth, differentiation, and dysmorphogenesis. Statistical analyses included the Mann-Whitney test and t-test. Effects and their statistical significance are outlined in Table 3-19. The Expert Panel identified no observed effects concentrations (NOECs) for each compound and they are listed in Table 3-20. In embryos treated with 200 mM ethylene glycol, significant effects included reduced yolk sac diameter, crown-rump length, protein content and differentiation score. A 47% rate of dysmorphogenesis was noted, and effects most commonly observed included defects in the head region, incomplete flexion, and unclearly shaped somites. Growth and differentiation were adversely affected in embryos treated with ≥3 mM glycolic acid. Rates of dysmorphogenesis were 25% and 88% in the 6 mM and 10 mM glycolic acid groups, respectively. Defects were most often noted in the head and shape (rotation) of the embryo; somites could not be counted in the 10 mM group. Similar effects were noted with the other metabolites that were about 3- to 10-fold more potent than glycolic acid (Table 3-18). As noted by study authors, embryotoxic levels of glycolic acid *in vitro* were consistent with glycolic acid blood levels observed following administration of developmentally toxic doses of ethylene glycol to rats. However, the embryotoxic concentrations of ethylene glycol and the other metabolites *in vitro* most likely exceeded blood levels that would be obtained with exposure to toxic doses of ethylene glycol.

Strengths/Weaknesses: The whole embryo culture technique is a good choice to examine the direct embryotoxic potential of ethylene glycol and its various metabolites due to the embryo's very limited ability to further metabolize these compounds. Six different compounds were tested *in vitro* with multiple doses of each compound used. However, dose-response data were limited with glyoxal, glyoxylic acid and oxalic acid since only two doses of each of these compounds were used. For most of the treatments, adequate numbers of embryos (15-19) were evaluated; however, in some groups only a few embryos (5-7) were examined. All data from control embryos were pooled, and the same control data are presented for each compound. Although it was not stated that the developmental stage of the embryos was controlled at the beginning of the experiment, a shortened breeding period (2 hours) was used, so the developmental stage of all embryos should have been similar at the beginning of culture. Insufficient detail was presented to determine if the statistical analysis was appropriate.

Utility (Adequacy) for CERHR Evaluation Process: The data are somewhat useful for the CERHR evaluative process in that the data on ethylene glycol and glycolic acid basically confirm the data presented by Carney et al. *(90)*. Both studies found no developmental toxicity at doses of ethylene glycol that could be reasonably achieved *in vivo*, and glycolic acid at 3 or 12.5 mM was embryotoxic. These concentrations of glycolic acid are within the range achieved after a teratogenic dose of ethylene glycol *in vivo* [~8 mM; Carney et al. *(45)*]. The concentrations of the other metabolites are higher than would be anticipated *in vivo* after a toxic exposure to ethylene glycol. The utility of the rest of the data to the CERHR evaluative process is limited by the pooling of control data and questionable statistical analysis.

Table 3-19. *In Vitro* Experiment With Ethylene Glycol and Metabolites, Klug et al. *(93)*.

Chemical: Concentration in mM (number embryos evaluated)	Effects				
	Yolk sac (mm)	**Crown-rump length (mm)**	**Somites (n)**	**Protein (μg/embryo)**	**Score**
Control (38)	**4.47**	**3.57**	**26**	**188**	**36**
Ethylene glycol:					
50 (7)	4.20	3.36	25	180	35
100 (15)	4.20*	3.48	25	175	35
200 (15)	4.08**	3.36*	26	142**	33**
Glycolic acid:					
1 (5)	3.90**	3.66	26	219	37
3 (17)	4.44	3.42**	26	149*	35
6 (16)	4.20*	2.91**	24** (3)[a]	106**	32**
10 (16)	3.21**	2.40*	ND	68**	25**
Oxalic acid:					
1 (16)	4.68	3.42*	26	193	36
2 (16)	3.90**	3.00**	23** (3)[a]	136**	25**
Glycolaldehyde:					
0.03 (19)	4.56	3.66	26	163	36
0.1 (16)	4.50	3.72	26	205	37*
0.2 (18)	4.05**	3.39*	25	161*	36
0.3 (5)	3.00**	2.70**	21 (1)[a]	114**	29**
Glyoxal:					
3 (16)	4.5	3.66	27*	188	37*
6 (18)	3.60**	2.79**	26	113**	30**
Glyoxylic acid:					
0.3 (19)	4.32*	3.60	26	190	36
1 (17)	2.88**	2.52**	24** (8)[a]	77**	29**

Notes: *=0.01≤p≤0.05, **p≤0.01
[a]Number in parentheses indicates the numbers of embryos in which somites could be counted.
ND=Could not be determined.

Table 3-20. Effect Levels (Selected by Expert Panel) for *In Vitro* Exposure to Ethylene Glycol and Its Metabolites by Klug et al. *(93)*.

Chemical	NOEC (mM)*	LOEC (mM)
Ethylene glycol	≥100<200	200
Glycolic acid	≤1	3
Oxalic acid	<1	1
Glycolaldehyde	0.1	0.2
Glyoxal	3	6
Glyoxylic acid	≤0.3	1

Carney et al. *(42*) conducted a study to determine the dose-rate effects of ethylene glycol on developmental toxicity. Pregnant CD (CRL: CD (SD) IGS BR) rats were randomly assigned to groups that received 0, 1,000, or 2,000 mg/kg bw/day ethylene glycol (99.91% purity) on gd 6-15. Eighteen rats/group received bolus doses by sc injection, while 16-20 rats/group were slowly and continuously dosed through sc pumps. Distilled water was the vehicle control. The lower dose was approximately equal to the LOEL observed in the Neeper-Bradley et al. *(77*) study in rats, and the high dose was the maximum amount that could be delivered by sc pump. The sc route was previously demonstrated to be effective in producing ethylene glycol-induced developmental toxicity by Khera *(89*). Concentrations of dosing solutions were confirmed to be within 100-102% of target values. Blood levels of ethylene glycol and glycolic acid were measured by GC/MS in rats treated by bolus injection or continuous infusion (3 rats/group) on gd 7, 9, 12, and 15. Blood samples were taken from the bolus exposure group at 3 hours following treatment, but it is not clear when blood was taken from animals treated by continuous infusion. Maternal toxicity was assessed by observing clinical signs and measuring body weight gain, food consumption, and urinalysis parameters on gd 7 and 15, and liver and kidney weight on gd 21. On gd 21, dams were sacrificed and necropsied. Implantation sites were examined, corpora lutea were counted, and uteri of apparently non-pregnant rats were stained with 10% sodium sulfide to determine if early resorptions occurred. All fetuses were sexed, weighed, and examined for viability and external malformations. About half of the fetuses were stained with Alizarin Red S and examined for skeletal malformations. Heads from the remaining fetuses were preserved in Bouin's solution and sectioned to evaluate craniofacial defects. Statistical analyses included Bartlett's test for equality of variances, ANOVA, Dunnett's test, the Wilcoxon Rank-Sum test with Bonferroni's correction, the Fisher exact probability test with Bonferroni's correction, and binomial distribution test.

In the 1,000 and 2,000 mg/kg bw/day bolus groups, the mean blood levels of ethylene glycol (9.5 mM and 21.9 mM, respectively) and glycolic acid (3.3 mM and 6.3 mM, respectively) were higher compared to mean ethylene glycol (2.3 mM and 4.3 mM, respectively) and glycolic acid (0.1 mM and 1.0 mM, respectively) blood levels in rats treated by sc pump. Maternal and fetal toxicity were evident in the groups treated with bolus doses. Dams in both ethylene glycol bolus groups had reddish-colored urine, changes in urinalysis parameters (i.e., non-significant increases in protein, bilirubin, and blood), and increased relative and absolute liver weight. Additional effects noted in dams of the 2,000 mg/kg bw/day bolus group included uncoordinated gait, reduced activity, decreased bodyweight gain on gd 6-9 and 6-21, reduced food intake on gd 6-9, and increased relative kidney weight (no histopathology conducted). In litters from the 1,000 mg/kg bw/day bolus group, a non-significant increase in extra vertebrae and ribs and significantly reduced ossification of several axial skeleton structures were considered to be treatment-related by authors. Also noted in litters of the 2,000 mg/kg bw/day group were significant increases in axial skeleton malformations and significantly reduced fetal bodyweight. No dose-related evidence of maternal or fetal toxicity was noted in rats treated with infusion pumps. According to the study authors, increased bodyweight gain in the 2000 mg/kg bw/day infusion pump group was likely due to increased litter size. When terminal bodyweights were corrected for gravid uterine weight, the 2000 mg/kg bw/day infusion group did not differ from controls. The study authors concluded that "These data support the hypothesis that dose-rate is a critical determinant of ethylene glycol-induced developmental toxicity and demonstrate how bolus dosing studies can greatly overestimate the risk to humans of ethylene glycol exposures, which typically involve low doses and/or slow dose-rates." **[Bolus dosing may, however, provide useful data for predicting effects resulting from poisonings.]**

Strengths/Weaknesses: This study is a well-conducted, GLP study performed in accordance with regulatory guidelines and standard practices using appropriate numbers of animals and statistical analyses. Fetuses were evaluated for signs of developmental toxicity using appropriate methods. The purity of the test chemical was known, and the doses administered to animals were analyzed and were found to be 100-102% of their target.

Utility (Adequacy) for CERHR Evaluation Process: The data presented in this study is useful for the CEHRH evaluative process and demonstrates that developmental toxicity is more closely related to blood concentrations of glycolic acid than to administered dose of ethylene glycol. The data presented in this study are consistent with previous reports. The malformations observed in this study after bolus administration of 2000 mg/kg (primarily skeletal defects – fused or missing ribs, decreased ossification of vertebral centra) were similar to defects reported by Khera *(89)* after subcutaneous administration of 2800 mg/kg; glycolic acid levels in these animals were approximately 6.3 mM. No malformations were observed when the same dose of ethylene glycol was administered by subcutaneous pump; blood levels of glycolic acid in these animals were 1.0 mM. Previous *in vitro* studies had shown embryotoxicity of glycolic acid at 3-6 mM *(93)* or 12.5 mM *(90)*. Therefore, these data support the hypothesis that the proximate teratogen is glycolic acid. A detailed evaluation regarding toxicokinetic parameters of this study is included in Section 2.1.3.2.

Carney et al. *(34)* conducted preliminary and probe studies to determine if differences in susceptibility to ethylene glycol-induced toxicity in rabbits and rats is due to differences in toxicokinetics or in the amount of toxic metabolites reaching the embryo. In both experiments conducted according to GLP, isotopically normal and/or $^{13}C_2$-ethylene glycol (99.7% purity and 96.7% purity, respectively) were administered in aqueous solution by gavage to pregnant 5-6 month old New Zealand White rabbits and 8-9 week old Sprague-Dawley rats. Doses were based on effects observed in teratogenicity studies *(24, 68)*, as described in Section 3. Dosing solutions were analyzed to verify target concentrations, stability, and homogeneity. Levels of ethylene glycol and its metabolites in body fluids were analyzed by GC/MS. Because small numbers of animals were used, results were only evaluated by descriptive statistics, such as mean±SD.

In the preliminary study, three pregnant rabbits were gavage dosed with 2,500 mg/kg ethylene glycol on gd 9, and blood samples were collected at time intervals between 0.5 and 24 hours after dosing for an analysis of acid-base balance and ethylene glycol, glycolic acid and oxalic acid levels. None of the rabbits experienced metabolic acidosis as determined by blood pH, PCO_2, or bicarbonate levels. Absorption of ethylene glycol was rapid with peak blood levels of parent compound occurring at 1 hour post dosing. Glycolic acid levels increased slowly with no clear C_{max} in most animals. Authors stated that blood glycolic acid levels remained constant and within peak values at 4-12 hours post dosing. Blood oxalic acid levels were not consistently increased over baseline levels. The study authors noted that in contrast to rats, rabbits do not develop metabolic acidosis with exposure to 2,500 mg/kg ethylene glycol and experience a slower increase in blood glycolic acid levels with peak blood levels occurring at approximately 1 and 4-12 hours, respectively.

A probe study was next conducted by Carney et al. *(34)* to compare levels of ethylene glycol, glycolic acid, and oxalic acid in maternal blood and extraembryonic fluids (EEF) in rats and rabbits. Gavage doses of 500 and 2,500 mg/kg bw were administered to six pregnant rats/dose group on gd 10 and six pregnant rabbits/dose group on gd 9. Authors stated that gd 10 in rats and gd 9 in rabbits represent equivalent periods (early somite stages) of development. Blood and extraembryonic fluid (pooled by litter) were collected at 1 hour post dosing in 3 rats and rabbits/dose group, at 3 hours post dosing in 3 rats/dose group, and at six hours post dosing in 3

rabbits/dose group. The first collection period was based on the estimated time of peak ethylene glycol concentrations, and the second collection period was based on estimated time of peak glycolic acid levels in each species. Concentrations of ethylene glycol and glycolic acid in maternal blood and extraembryonic fluids are listed in Table 3-21. Maternal blood levels of ethylene glycol were very similar in rats and rabbits at both doses and time periods leading study authors to conclude that oral absorption does not account for the different sensitivity in ethylene glycol toxicity in these two species. Levels of ethylene glycol in extraembryonic fluid were about twice as high in rats at 1 hour post dosing but were approximately equal at 3 and 6 hours post-dosing in rats and rabbits, respectively. In rabbits, glycolic acid concentrations were 3-18 fold lower in blood and 4-38 fold lower in extraembryonic fluid compared to rats. The authors suggested this may represent a quantitative difference in metabolism or elimination between the two species. Levels of glycolic acid in extraembryonic fluid exceeded maternal blood levels in rats, but in rabbits levels of glycolic acid were usually lower in extraembryonic fluid than in maternal blood. According to authors, glycolate ion "trapping" appears to occur in the rat but not rabbit conceptus. "Trapping" is a term used to describe passage of the non-ionized form of a weak acid (present at low blood pH) across cell membranes to the extraembryonic fluid; the higher pH of the extraembryonic fluid causes the weak acid to become ionized and thus unable to diffuse back across cell membranes. Levels of oxalic acid were near or lower than background levels, but were slightly elevated in EEF from rats dosed with 2,500 mg/kg bw. In cases where oxalic acid was detected, levels in rabbits were about half of those in rats. Study authors postulated that oxalic acid may be an additive factor in rat developmental toxicity at very high doses.

It was noted by Carney et al. *(34*) that absolute blood values of ethylene glycol and its metabolites were about 3-fold higher in the preliminary toxicokinetic study compared to values obtained at comparable time periods in rabbits from the probe study. They determined that differences were most likely due to variability between animals and slight differences in analytical conditions.

Strengths/Weaknesses: This study was conducted under GLP conditions with test material of known purity. Doses of ethylene glycol that had previously been examined *in vivo* were used. Although the study was well conducted, very small numbers of animals were used, and only descriptive statistics were presented.

Utility (Adequacy) for CERHR Evaluation Process: These data are very interesting, but due to their preliminary nature, they are not very useful for the CEHRH evaluative process. They do present a plausible explanation for differences in developmental toxicity between rats and rabbits. These results should be followed up by a more thorough experimentation.

Table 3-21. Comparison of Maternal Blood and Extraembryonic Fluid Levels of Ethylene Glycol and Glycolic Acid in Rats and Rabbits Dosed With Ethylene Glycol, Carney et al. *(34)*.

mg/kg Ethylene Glycol	Hours Post Dose	Ethylene Glycol Level (mM)*		EEF/MB for Ethylene Glycol	Glycolic Acid Level (mM)*		EEF/MB for Glycolic Acid
		MB	EEF		MB	EEF	
Rat:							
500	1	7.48	7.56	1.01	1.50	2.60	1.74
500	3	3.97	3.55	0.89	2.20	3.96	1.80
2500	1	27.48	30.66	1.12	4.87	6.47	1.33
2500	3	18.22	26.04	1.43	8.89	15.33	1.72
Rabbit:							
500	1	7.01	4.08	0.58	<0.07	<0.07	No data.
500	6	2.87	4.66	1.62	0.73	1.04	1.42
2500	1	26.93	16.72	0.62	0.56	0.18	0.32
2500	6	18.58	28.79	1.55	3.62	2.67	0.74

MB=Maternal Blood
EEF=Extraembryonic fluid
Ethylene Glycol=ethylene glycol
Glycolic Acid=glycolic acid
*Study authors have stated that this data should not be considered definitive.

3.3 Utility of Data

3.4 Summary

Human Data

No human data were identified.

Experimental Animal Data

There are sufficient data to conclude that prenatal oral exposure to high doses of ethylene glycol cause developmental toxicity in rats and mice. The results of the key studies are summarized below and outlined in Table 3-22. The Expert Panel noted that ethylene glycol was delivered by gavage in the majority of oral exposure studies, and that bolus doses do not represent expected human environmental exposures. Developmental toxicity was not observed in rabbits orally exposed to ethylene glycol at doses associated with severe maternal toxicity. Aerosol studies are inconclusive in determining if developmental toxicity occurs by this exposure route due to oral exposure associated with grooming. Dermal exposure was not associated with developmental toxicity in studies with mice.

Oral Exposure

Mice. Studies conducted by Price et al. *(69)* and Tyl and Frank *(76)* demonstrate that ethylene glycol is a developmental toxicant in CD-1 mice following gavage exposure to ≥500 mg/kg bw/day on gd 6-15. Exposure to 500 mg/kg bw/day increased the numbers of litters containing pups with malformations with no specific malformation elevated to a level of statistical significance. Malformations that primarily affected the axial skeleton were increased in litters exposed to ≥750 mg/kg bw/day. At a dose of 3000 mg/kg bw/day, neural tube closure and craniofacial defects were observed. Additional evidence of developmental toxicity included reduced fetal bodyweight (≥750 mg/kg bw/day) and reduced numbers of live fetuses (≥3000 mg/kg bw/day). The developmental NOAEL for mice was identified as 150 mg/kg bw/day. Maternal toxicity was limited to decreased absolute liver weight at doses of ≥1500 mg/kg bw/day in the Price et al. *(69)* study, while no evidence of toxicity was noted in dams exposed to 1500 mg/kg bw/day in the Tyl et al. *(76)* study.

Developmental toxicity observed in mouse continuous reproductive breeding studies *(94, 95)* were consistent with those observed in prenatal studies, although differences in study design and method of dosing preclude a direct comparison.

The Panel concluded there is sufficient evidence in mice that gavage exposure to ≥500 mg/kg bw/day ethylene glycol on gd 6-15 causes developmental toxicity in the form of malformations.

Rats. Studies conducted by Price et al. *(69)* and Neeper-Bradley et al. *(77, 78)* demonstrate that high doses of ethylene glycol administered by gavage on gd 6-15 cause developmental toxicity in Sprague-Dawley rats. Skeletal malformations were found to be the most sensitive endpoint. In the Neeper-Bradley et al. *(77, 78)* study, an increase in axial skeleton malformations were noted in groups dosed with ≥1000 mg/kg bw/day group. In contrast, no skeletal malformations were noted in offspring of rats dosed with 1250 mg/kg bw/day in the Price et al. *(69)* study; an increase in skeletal malformations was noted in groups dosed with ≥2500 mg/kg bw/day. The Expert Panel noted that sacrifice of animals on different days (gd 20 for the Price et al. study and gd 21 for the Neeper-Bradley study) complicates direct comparison of the two studies. Additional evidence of developmental toxicity in the Price et al. *(69)* and Neeper-Bradley et al. *(77, 78)* studies included reduced fetal bodyweights (1000-5000 mg/kg bw/day), increased soft tissue and external malformations consisting primarily of neural tube closure and craniofacial defects (2500-5000 mg/kg bw/day), reductions in live fetuses (2500-5000 mg/kg bw/day) and increased postimplantation loss (5000 mg/kg bw/day). The developmental toxicity NOEL was identified as 500 mg/kg bw/day *(77, 78)*. Effects noted in dams by Price et al. *(69)* and Neeper-Bradley et al. *(77, 78)* included reduced bodyweight gain with no effect on bodyweight corrected for gravid uterine weight (≥1250 mg/kg bw/day), increased kidney weight and water intake (≥2500), and decreased liver weight (5000 mg/kg bw/day). There were no kidney lesions observed in dams at doses up to 2500 mg/kg bw/day. The maternal NOEL was identified as 1000 mg/kg bw/day *(77, 78)*.

In a dietary study where Fischer 344 rats received 40-1000 mg/kg bw/day ethylene glycol on gd 6-15, there was no increase in malformed fetuses *(79)*. A significantly increased incidence of fetuses but not litters with poorly or unossified vertebrae was considered by the study author to be evidence of delayed fetal maturation and suggestive of minimal toxicity. However, noting the absence of bodyweight effects or other consistent changes in skeletal integrity, the Expert Panel concluded that the 1000 mg/kg bw/day dose should be classified as a NOAEL and not a LOAEL.

Price et al. *(81*) evaluated postnatal development in a study where Sprague-Dawley rats were gavage dosed with ethylene glycol on gd 6-20 and allowed to litter. Gestational length was increased at doses ≥1250 mg/kg bw/day. An increase in pup mortality on pnd 1-4 and reduced pup weight gain on pnd 1-22 was noted at the 2250 mg/kg bw/day dose level. There were no effects on neurobehavioral tests (i.e. exploratory behavior and visual discrimination tests) or developmental landmarks (i.e. incisor eruption, vaginal opening, testes decent, or wire grasping skills) at doses up to 2250 mg/kg bw/day. Malformations in pups were consistent with those observed in prenatal studies.

A study examining fetuses and pups of rats gavage dosed with 2500 mg/kg bw/day ethylene glycol on gd 6-15, demonstrated fewer skeletal variations and malformations in pups examined on pnd 63 versus fetuses and pups examined at various time points up to pnd 21 *(82*). The study suggests that many ethylene glycol-induced skeletal changes, most which are variations, are reversible.

The Expert Panel concluded there is sufficient evidence in rats that gavage exposure to ≥1000 mg/kg bw/day ethylene glycol on gd 6-15 causes developmental toxicity in the form of skeletal malformations.

Rabbits. A study conducted by Tyl et al. *(68*) demonstrated no developmental toxicity in rabbits following gavage exposure with up to 2000 mg/kg bw/day on gd 6-19, as noted by a lack of teratogenicity, prenatal mortality, and effects on fetal growth. Severe maternal toxicity was observed at 2000 mg/kg bw/day and included a 42% death rate, increased early delivery, renal lesions, and renal oxalate crystals. The maternal and fetal NOAELs were identified as 1000 and 2000 mg/kg bw/day respectively.

The Expert Panel concluded that data are sufficient to demonstrate a lack of developmental toxicity in rabbits following gavage of does with up to 2000 mg/kg bw/day ethylene glycol on gd 6-19.

Inhalation Exposure

Mice. Tyl *(83*) exposed CD-1 mice to ethylene glycol aerosols by whole body inhalation on gd 6-15 and found increased malformations at ≥1000 mg/m^3. However, it was noted that ethylene glycol was deposited on the fur of animals and that oral ingestion through grooming could account for a large percentage of the total dose. In order to examine the role of ethylene glycol exposure alone, Tyl et al. *(84, 85*) repeated the study, exposing the mice to ethylene glycol by nose-only. The only developmental effects were reduced ossification at ≥500 mg/m^3 and increased fused ribs at ≥1000 mg/m^3. The Expert Panel agreed with study author observations that the studies were confounded by exposure through ingestion in both the whole-body and nose-only study and stress induced by restraint in the nose-only study.

The Expert Panel concluded that the data are insufficient to determine if inhalation of ethylene glycol causes developmental toxicity in mice.

Rats. A whole-body inhalation study was conducted by Tyl *(83*) in Sprague-Dawley rats. However as discussed above for mice, the findings of the study were confounded by oral intake from grooming of fur containing ethylene glycol. The Expert Panel concluded that the data are insufficient to determine if inhalation of ethylene glycol causes developmental toxicity in rats.

Dermal Exposure

Tyl *(74)* exposed CD-1 mice to ethylene glycol by the dermal route for 6 hours/day on gd 6-15 and found no evidence of malformations, increased prenatal mortality, or delayed growth at doses up to 1677 mg/kg bw/day. The only fetal effects observed at the highest dose of 3549 mg/kg bw/day were reduced ossification of skull bones and phalanges. The maternal and fetal NOELs were identified as 3549 mg/kg bw/day.

The Expert Panel concluded that data are sufficient to demonstrate a lack of developmental toxicity in mice following dermal exposure with up to 3549 mg/kg bw/day ethylene glycol for six hours/day on gd 6-15.

Mechanistic Issues

Variability Across Dose Routes and Dose Rate Effects. As noted above, ethylene glycol was more toxic to the conceptus when administered by the oral versus dermal route. Toxicokinetic studies reviewed in Section 2, demonstrated that in contrast to rapid and complete absorption through the oral route, dermal exposure in rats and mice is slow and incomplete *(30-32*).

Carney et al. *(42*) compared blood levels of ethylene glycol and glycolic acid and developmental toxicity in Sprague-Dawley rats administered 1,000-2,000 mg/kg bw/day ethylene glycol on gd 6-15 by bolus sc injection or slow and continuous infusion by an sc pump. Compared to bolus injection, continuous infusion resulted in lower blood levels of ethylene glycol and glycolic acid and no evidence of developmental toxicity. The study suggested that a peak glycolic acid concentration of 2 mM is needed to produce developmental toxicity. However, the AUC for ethylene glycol was not measured and it is therefore not known if difference in toxicity was partly due to varying bioavailability of ethylene glycol for the two dosing methods.

Proximate Teratogen. A series of *in vivo* and *in vitro* studies sought to identify the proximate developmental teratogen associated with ethylene glycol exposure. *In vitro* studies *(88, 93*) demonstrated that ethylene glycol produced dysmorphogenesis in 9.5-10.5 day old rat embryos only at concentrations (≥200 mM) greatly exceeding blood levels (14-57 mM) observed with in vivo exposures of rats to teratogenic concentrations of ethylene glycol *(30, 32, 33*). These studies suggested that either a metabolite or metabolic acidosis may be responsible for developmental toxicity. Coadministration of sodium bicarbonate to rats administered 2800 and 3333 mg/kg bw ethylene glycol by sc injection on gd 11 reduced metabolic acidosis effects in dams and decreased the incidence of skeletal defects in fetuses compared with administration of ethylene glycol alone *(89*). In Sprague-Dawley rats gavage-dosed with ≥300 mg/kg bw/day glycolic acid on gd 7-21, *(92*) fetal malformations of the axial skeleton were consistent with those observed in rats dosed with ethylene glycol at ≥1000 mg/kg bw/day ethylene glycol *(77*). In vitro exposure of 9.5-10.5-day-old rat embryos to glycolic acid *(90, 93*) resulted in dysmorphogen-esis at media concentrations (3-12.5 mM) within the ranges of glycolic acid blood levels (4.8-8.8 mM) observed in rats administered teratogenic doses of ethylene glycol *(33, 45*). Though other metabolites (i.e. oxalic acid, glycolaldehyde, glyoxal, and glyoxylic acid) were found to be more potent than ethylene glycol, in vitro developmental toxicity in rat embryos was only observed at media concentrations greatly exceeding levels observed in *in vivo* studies *(93*). In an in vitro study, dysmorphogenesis in gd 10.5 rat embryos treated with 12.5 mM sodium glycolate (pH=7.42) was similar but lower in magnitude than in embryos treated with 12.5 mM glycolic acid (pH=6.74) *(96*). In a subsequent study, Sprague-Dawley rats were treated with ethylene glycol, glycolic acid, or sodium glycolate at levels that produced identical peak levels of glycolate (8.4-8.8 mM), but a three-fold higher AUC with ethylene glycol versus glycolic acid or sodium

glycolate administration; *(45*) metabolic acidosis occurred only in groups treated with ethylene glycol or glycolic acid. Skeletal defect patterns were similar in the ethylene glycol and glycolic acid groups with a higher incidence in the ethylene glycol group; increased glycolic acid AUC with ethylene glycol dosing is a possible reason for the higher incidence and greater severity of defects. Only a few skeletal variations were observed in the sodium glycolate group.

According to the Expert Panel, mechanistic data suggest that unmetabolized ethylene glycol is not likely to be the proximate teratogen in rodents. Glycolic acid in combination with resulting metabolic acidosis and possibly maternal toxicity is the most likely cause of developmental toxicity following exposure of rodents to ethylene glycol.

Interspecies Variability. A preliminary study suggested metabolic differences between rats and rabbits following exposure to a high dose of ethylene glycol, *(34*) but the preliminary nature of the data do not allow definite conclusions to be made about interspecies differences. Because a metabolite, i.e., glycolic acid, appears to be the proximate teratogen, the documented species differences in the ontogeny of enzymes capable of metabolizing ethylene glycol to this metabolite may play a role in species-specific susceptibility to ethylene glycol developmental toxicity.

Table 3-22. Summary of Key Developmental Toxicity Studies.

Doses (mg/kg bw/day)	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
750 1500 3000	gd 6-15, gavage	CD-1 Mouse	**Dams:** **Maternal NOAEL=750** **1500:** ↓Bodyweight gain and ↓liver weight **3000:** ↓Bodyweight gain and ↓liver weight **Fetuses:** **750:** ↓Bodyweight/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑litters with skeletal malformations **1500:** ↓Bodyweight/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑litters with skeletal malformations **3000:** ↓Live fetuses/litter, ↓bodyweight/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑litters with external, visceral, and skeletal malformations	Price et al. *(69)*
50 150 500 1500	gd 6-15, gavage	CD-1 Mouse	**Dams:** **Maternal NOEL=1500** **Fetuses:** **Fetal NOEL=150** **500:** ↑Litters with malformations **1500:** ↓Bodyweight/litter, ↑litters with malformations, ↑litters with skeletal malformations	Tyl and Frank *(76)*
1250 2500 5000	Gd 6-15, gavage	CD Rat	**Dams:** **1250:** ↓Bodyweight gain **2500:** ↓Bodyweight gain, ↑relative (to body weight) kidney weight, ↑water intake **5000:** ↓Bodyweight gain, ↓ liver weight, ↑relative (to body weight) kidney weight, ↑water intake **Fetuses:** **Fetal NOAEL=1250[a]** **2500:** ↓Live fetuses/litter, ↓bodyweight/litter, ↑malformed fetuses/litter, ↑ litters with malformed fetuses, ↑litters with skeletal malformations **5000:** ↑Postimplantation loss/litter, ↓live fetuses/litter, ↓bodyweight/litter, ↑malformed fetuses/litter, ↑litters with malformed fetuses, ↑ litters with external malformations, ↑litters with visceral malformations, ↑litters with skeletal malformations	Price et al. *(69)*
150 500 1000 2500	gd 6-15, gavage	CD Rat	**Dams:** **Maternal NOEL=1000** **2500:** ↓Bodyweight gain, ↑water intake, ↑absolute and relative (to body weight)	Neeper-Bradley et. al. *(77, 78)*

Doses (mg/kg bw/day)	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
			kidney weights, ↑relative (to body weight) liver weight **Fetuses:** **Fetal NOEL=500 mg/kg bw/day** **1000:** ↓Bodyweight/litter, ↑litters with skeletal malformations **2500:** ↓Bodyweight/litter, ↑litters with external, visceral, skeletal, and total malformations,	
40 200 1000	gd 6-15, diet	Fischer 344 Rat	**Dams:** **Maternal NOAEL=1000** **Fetuses:** **Fetal NOAEL=1000**[a]	Maronpot et al. *(79)*
250 1250 2250	gd 6-20, gavage	CD Rats	**Dams:** **Maternal NOAEL=250** **1250:**↑Gestational length, ↑renal lesions **2250:** ↓Bodyweight gain; ↑gestational length; ↑renal lesions; ↑absolute and relative (to body weight) kidney weights, ↓ absolute and relative (to body weight) uterine weight, **Pups:** **Pup NOAEL=1250** **2250:** ↓Live litter size and ↑ pup mortality on pnd 1 and 4, ↓postnatal weight gain, ↓kidney weight (M), ↓brain weight, ↑skeletal malformations	Price et al. *(81)*
100 500 1000 2000	gd 6-19, gavage	New Zealand White Rabbit	**Dams:** **Maternal NOAEL=1000** **2000:** ↑Renal crystals and lesions and death **Fetuses:** **Fetal NOAEL=2000**	Tyl et al. *(68)*
404 1677 3549	6 hours/day on gd 6-15, dermal	CD-1 mice	**NOEL=3549 mg/kg bw/day for both dams and fetuses**	Tyl *(74)*

[a]The Expert Panel's selection of a NOAEL is higher than the study authors selection.

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4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

No human reproductive toxicity data were identified.

4.2 Experimental Animal Data

Using a continuous breeding protocol, Lamb et al. *(95)* investigated the reproductive function of mice exposed to ethylene glycol. Ethylene glycol (99.6% purity) was administered in drinking water to male and female COBS Crl:CD-1 (ICR) BR outbred albino mice, 20/dose/sex, at concentrations of 0.25, 0.5, or 1.0% w/v. Forty control mice/sex were exposed to the vehicle. The approximate doses were stated by the authors to be 410, 840, and 1640 mg/kg/day. Dose selection was based upon results of a range-finding study and the goal was to achieve no toxicity at the lowest dose and a 10% reduction in body weight at the highest dose. Concentrations of ethylene glycol in dosing solutions were verified. At 11 weeks of age, mice were continuously treated with the chemical during a one week pre-mating period, a 14-week cohabitation period, a 3-week segregation period, and at least until weaning of the offspring born during the 3-week segregation period. Newborn litters were examined, sexed, and weighed. With the exception of the last litter, the pups were immediately killed, allowing the pairs to breed again. F_1 litters born after separation of males and females were reared and weaned; the treated F_1 continued to receive ethylene glycol throughout their lifetime. Twenty F_1 pairs/group from the control and high dose group were retained for subsequent mating within groups at 70 days of age. The F_1 mating pairs were cohabitated until a copulatory plug was found or seven days had elapsed, whichever came first. F_2 pups from 1 litter/pair were examined, sexed, weighed, and then discarded. F_1 mice were necropsied after the mating trial and weights were measured for liver, brain, pituitary, and male and female reproductive organs. **[Histopathology findings in reproductive organs were not reported.]** Each pair of mice was considered the experimental unit. Reproductive data were evaluated by the Chi-Square test for homogeneity and or the Fisher's Exact test. Pup and litter data were evaluated by Chi-Square approximation to the Kruskal-Wallis test, Fisher's Exact test, and the Mann-Whitney U test.

Results of the testing conducted in F_0 rats is listed in Table 4-1. Thirty-eight pairs of F_0 mice in the control group and 18-20 pairs in treatment groups survived. No treatment related effects were observed on body weight, clinical signs, or water consumption at any dose level. The authors reported a slight but statistically significant decrease in number of litters/fertile pair ($p<0.01$), mean number of live pups/litter ($p<0.05$), and mean live pup weight ($p<0.01$) in the 1.0% ethylene glycol group. Neither the 0.25 or the 0.50% dose groups were significantly affected.

In the F_1 generation, 16 control pairs and 11 high-dose pairs produced litters. There were no significant differences in fertility, live litter size, or live pup weight between the control and 1.0% ethylene glycol groups. A number of F_1 animals in the 1640 mg/kg bw/day group were noted to have unusual facial features. Further examination of the skeleton by staining with alizarin red in 4 mice/sex/group in the control and high dose group revealed a pattern of skeletal defects affecting the skull, sternebrae, ribs and vertebrae in both sexes of the high dose group.

Strengths/Weakness: This is a well designed study that used adequate numbers of animals and examined reproductive function in two generations. Limitations of the study include examination of reproductive function only in control and high dose F_1 animals, no histopathology in reproductive organs, and no sperm measurements.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for demonstrating no effects on reproductive function in mice at doses up to 0.5% (840 mg/kg bw/day). Exposure to 1.0% (1640 mg/kg bw/day) resulted in a minor effect on fertility (a slight decrease in the number of F_1 litters/pair of F_0 mice) and findings that were most likely developmental effects (reduced numbers of live F_1 pups/litter, decreased F_1 pup weight, and facial and skeletal malformations). No reproductive effects were observed in the high dose F_1 mice.

Table 4-1. Major Effects Observed in a Continuous Breeding Study in CD-1 mice by Lamb et al. *(95)*.

Effect	**Dose in % (mg/kg bw/day)**			
	0	**0.25 (410)**	**0.5 (840)**	**1.0 (1640)**
No. F_1 Litters/Pair of F_0	4.9	4.7	4.9	4.5**
No. F_1 Live Pups/Litter	10.8	10.4	10.5	10.2***
Live F_1 Pup weight (g)	1.63	1.64	1.58	1.53**
NOELs				
Protocol: Reproductive function studied in 20-40 F_0 mice given water with 0, 0.25, 0.5, or 1.0 ethylene glycol and 19-20 F_1 mice given water with 0 or 1.0% ethylene glycol. **Notes:** *=p<0.001, **=p<0.01, ***=p<0.05 No effects were seen on F_0 and F_1 bodyweight or clinical signs and F_1 fertility, live litter size, or F_2 live pup weight.				

Gulati et al. *(94, 97)* continued and extended the continuous breeding study reported by Lamb et al. *(95)* by testing a higher dose level and looking at additional endpoints. This study was conducted according to Good Laboratory Practices using ethylene glycol (99.6% purity) administered in drinking water at concentrations of 0, 0.5, 1.0, and 1.5% (w/v) to COBS Crl: CD-1 (ICR) BR outbred albino mice. Concentrations of dosing solutions were verified. The authors estimated doses at 0, 897, 1798, and 2826 mg/kg bw/day, respectively. At 11 weeks of age, 40 control mice/sex and 20 treated mice/sex were exposed during a one week premating period. Males and females were then paired 1:1 within dose groups and exposures continued through a 14-week cohabitation period, a 21-day separation period, and until weaning of the last litter. Pups born during the cohabitation period were examined, sexed, and weighed. With the exception of the last litter born, pups were discarded after examination so the parental animals could continue mating. F_1 litters born after separation of males and females (at the end of 14 weeks) were saved and nursed through weaning. When F_1 litters were weaned, a crossover mating trial was conducted in F_0 mice by breeding 20 male and female mice from the high dose group to 20 control mice/sex. The purpose of the trial was to determine whether one or both sexes were affected. F_1 mice from all dose groups continued to receive treatment and at 74 days of age, 20 males and females/dose were mated within treatment groups. In both the crossover and F_1 mating studies, animals mated until a vaginal plug was detected or 7 days passed; litters were examined, sexed, weighed, and discarded. Fertility data were analyzed using the Cochran-Armitage test, Fisher's exact test or chi-square test for homogenety. Pup and litter data were evaluated with the

Kruskall-Wallis test, Jonckheere's test, the Wilcoxon's rank-sum test, F-test, t-test, and/or Williams test. Table 4-2 lists the major findings of this study.

Thirty-eight pairs of F_0 mice from the control group and 14-20 pairs of mice from treatment groups were fertile. Fertility index at all doses did not differ from controls. However, female pup weight and pup weight adjusted for litter size were significantly reduced at all doses; live pups/litter were significantly reduced in the 1.5% group. The crossover mating study confirmed that there was no reduction in fertility in high dose males or females; the fertility rate was 50% in all groups. The only significant effect in the crossover study was reduced adjusted pup body weight in litters born to high dose females mated with control males. At the end of the study, estrous cycles were monitored for one week and sperm analyses, necropsies, and histopathological examination were performed in control and high dose F_0 mice. Organ sections were stained with hematoxylin-eosin for histopathological evaluation, but there was no mention of the fixation method. No treatment related effect on estrous cyclicity was noted, and histologic studies revealed no treatment related effects on ovary, uterus or vagina. In high dose F_0 males, sperm number in a sample from the cauda epidymis was similar to controls but the incidence of abnormal sperm increased and motility decreased significantly in the 1.5% group. Table 4-3 outlines the main histopathological findings for male reproductive organs. Testicular lesions that occurred at a higher frequency and severity in males treated with 1.5% ethylene glycol included degeneration of seminiferous tubules, loss of spermatozoa, spermatids, spermatogonia and spermatocytes, vacuolization of epithelial cells, and interstial cell hyperplasia. Epididymal lesions were also observed. Kidney lesions and oxyalate crystals were observed in the treated group. Body and absolute liver weight were significantly lower in males of the 1.5% dose group. There were no effects on male reproductive organ weights or female body, liver, and kidney weights. Blood calcium levels were not affected in any treatment group. **[The Expert Panel concluded that because effects on male reproductive organs and sperm parameters were only examined in controls and the 1.5% dose group, the data are inadequate to characterize these endpoints over this dose range.]**

A total of 13-18 pairs of F_1 mice/group were fertile. The treated F_1 mice showed no effect on mating or fertility index, number of live pups/litter, or sex ratio within litters. There was a significant decrease in adjusted live pup weight in all treatment groups with no evidence of a dose response relationship. At the end of the study, estrous cycles were monitored for one week and sperm analyses and necropsies were performed for all dose levels in F_1 mice, but histopathology was only conducted in control and high dose groups. Significant decreases were observed for absolute seminal vesicle and right testis weight at all dose levels and absolute epididymis weight in the 1.0 and 1.5% groups. Relative right testis and epididymis weights were significantly decreased in mice from the 1.0 and 1.5% dose groups. Sperm motility was significantly reduced at the 1.0 and 1.5% groups. Sperm count was decreased by about 20% at all doses; though there was no dose response, statistical significance was achieved at the 2 lower doses. A dose-related increase in abnormal sperm was not significant. The major histological findings for male reproductive organs are listed in Table 4-3. Histological examination in high dose mice revealed a higher incidence and severity of seminiferous tubule degeneration, epididymal lesions and interstitial cell hyperplasia in mice of the 1.5% dose group. No chemical-related histopathological lesions were observed in the reproductive tissues from high dose female mice and estrous cycles were not affected at any dose. Absolute liver weights were reduced in males and females of the 1.5% dose group. There were no lesions in livers or kidneys. An 18% mortality rate was observed in male mice from the 1.5% dose group prior to mating. Blood calcium levels were not affected in any treatment group. Facial abnormalities similar to those reported by Lamb et al. *(95)* were observed in F_1 mice from the 1.0 and 1.5% dose groups.

[The Expert Panel stated that at 1.5% ethylene glycol in drinking water, there was evidence of some degenerative changes in the testes and altered sperm parameters. While 85% of the treated animals revealed some degeneration of the seminiferous tubules, the control groups also exhibited a 57% incidence. There were no treatment-related microscopic lesions in the prostate gland or the seminal vesicles. There is no evidence of female reproductive toxicity at doses up to 1.5% in drinking water as noted by no effects on fertility, estrous cyclicity or histopathology of female reproductive organs (e.g., ovary, uterus, or vagina).]

Strengths/Weaknesses: Dose response limitations within the study protocol preclude establishing a NOAEL. In addition, reproductive parameters in the control group (e.g. degenerative changes in seminiferous tubules) were over 50% and hence render any scientific opinion inconclusive.

Utility (Adequacy) for CERHR Evaluation Process: The results of the Gulati et al. *(94)* study were essentially negative with respect to the effects of ethylene glycol on multigenerational findings in mice. According to Gulati et al. *(94)* ethylene glycol does not exhibit any significant toxicological effects upon mouse reproductive processes.

Table 4-2. Major Effects Produced by Ethylene Glycol in a Continuous Breeding Study in CD-1 mice *(94)*.

Effect	Dose in % (mg/kg bw/day)			
	0	**0.5 (897)**	**1.0 (1798)**	**1.5 (2826)**
F_0 Parents and F_1 Offspring:				
% Abnormal sperm in F_0	5.05	ND	ND	8.28***
% Motile sperm in F_0	94.3	ND	ND	80.6***
F_0 male bodyweight (g)	46.449	ND	ND	42.287***
No F_1 Litters/Pair of F_0	4.68	5.00***	4.85	4.43
F_0 male liver weight (g)	2.113	ND	ND	1.933***
No. Live pups/litter	11.81	11.64	11.99	9.99***
Adjusted (per litter size) live pup weight (g)	1.58	1.53***	1.48**	1.43**
Adjusted live pup weight (g) in crossover study	1.68	ND	ND	1.54*** /1.69[a]
F_1 Parents and F_2 Offspring:				
% Abnormal sperm in F_1	4.24	4.67	5.25	5.77
F_1 Sperm Count $x10^9$	1036	801***	855***	861
% Motile sperm in F_1	94.6	94.4	92.1***	84.1***
F_1 right testis weight (g)	0.140	0.124[b]***	0.119[b]**	0.120[b]***
F_1 seminal vesicle weight (g)	0.465	0.408***	0.409***	0.401***
F_1 epididymis weight (mg)	49.565	45.845	44.300[b]***	42.850[b]**
F_1 relative (to body weight) right testis weight (g)	0.137	0.125	0.120***	0.121***
F_1 relative (to body weight) epididymis weight (mg)	48.653	46.127	44.568***	43.212**
F_1 liver weight (g), male:	1.869	1.720***	1.739	1.692***
females:	1.662	1.730	1.630	1.518***
Adjusted (per litter size) live pup weight (g)	1.54	1.46***	1.46***	1.45***

Protocol: Reproductive function studied in 16-38 F_0 pairs/group, 16-20 F_0 treated x control pairs/group, and 20 F_1 pairs/group administered ethylene glycol through drinking water at 0, 0.5, 1.0, and 1.5%.

Notes: *=p<0.001, **=p<0.01, ***=p<0.05

ND=Not determined.

[a]Values for treated females x control male / control females x treated males.

[b]These values were listed in both Table 14 and 15 of the study but different levels of statistical significance were listed in the 2 tables.

No effects were noted for fertility index, estrous cycles, and histopathology or weights of female reproductive organs in either generation.

Table 4-3. Summary of Histopathological Effects in Male Mouse Reproductive Organs Caused by 1.5% Ethylene Glycol in Drinking Water *(94)*.

Effect: Severity[a]	Dose in % (mg/kg bw/day)	
	0[b]	1.5 (2826)[c]
F_0 Mice		
Seminiferous tubule degeneration:		
Minimal	11	11
Mild	1	3
Moderate	0	2
Severe	0	1
Interstitial Cell Hyperplasia:		
Minimal to moderate	0	3
Epididymal Lesions:		
Minimal to moderate	1	9
Epididymal Sperm Reduction:		
Moderate	0	4
F_1 Mice		
Seminiferous tubule degeneration:		
Minimal	7	10
Mild	1	0
Moderate	0	2
Interstitial Cell Hyperplasia:		
Minimal to mild	0	2
Epididymal Lesions		
Minimal to mild	0	4

Notes:
[a]Reported as total number of mice affected.
[b]21 F_0 and 20 F_1 mice were examined.
[c]20 F_0 and F_1 mice/generation were examined.
Statistical significance of effects was not reported.
There were no treatment-related effects on female reproductive organs.
See text for a description of kidney histopathology in F_0 males.

In a multi-generation study by Depass et al. *(98)* [also Woodside et al. *(99)*], ethylene glycol (99.82% purity) was added to the diet of male and female Crl:Fischer 344 rats to provide dosages of 0, 40, 200 or 1000 mg/kg bw/day. Ethylene glycol levels in diet were adjusted every two weeks to maintain constant dose levels. During the second and third week of lactation, ethylene glycol levels were reduced two and three-fold to adjust for large increases in food consumption that occur during this time. Authors verified that calculated doses based on nominal concentrations of ethylene glycol in diet were close to target doses. Preliminary studies indicated that the highest dose caused mild renal toxicity, and subsequently the dose selection was based upon the effects seen in the male since they appeared to be more susceptible than the female. Two groups of control rats were fed diets without ethylene glycol. Exposure of the F_0 males and females to ethylene glycol began at about 49 days of age (~7 weeks before mating) and was continued for 3 generations. At about 100 days of age, 10 males in each dosage group were

mated to 20 females in the same dosage group. The date of parturition and number of live and dead newborns was recorded for each litter. The litters were weighed at pnd 4 and 14 and individual pups were weighed at weaning (pnd 21). F_1 and F_2 rats were randomly selected for mating ensuring that each litter was represented. **[The exact number of F_1 and F_2 rats mated was not specified.]** Necropsies and histopathological examination were performed on 5 males and 5 females at each dosage level in the F_2 parents and F_3 weanlings. **[Methods for histological evaluation were not discussed.]** Continuous data were evaluated by ANOVA, Bartlett's test for homogeneity of variance, Duncan's multiple range test, and t-tests. Discontinuous data were analyzed by a multiple sum of ranks test, and frequency data by the x^2 test and Fisher's exact test. No effects on body weight gain or diet consumption were observed at any dose. Ethylene glycol treatment had no effect on fertility index, gestation index, gestation survival index and days from first mating to litter in the F_0-F_1, F_1-F_2 or F_2-F_3 generation. There was also no affect on postnatal pup weight gain. No histopathologic effects were observed in accessory sex glands, epididymis, testes, uterus, ovaries or kidneys of F_2 parents and or F_3 weanlings. **[The Expert Panel concluded that these data provide adequate evidence that continuous dietary exposure of rats to 40-1000 mg ethylene glycol/kg bw/day does not suppress the fertility index in three generations of rats mated once as mature adults. (Note a table was not prepared for this study since only negative results were obtained.)]**

Strengths/Weaknesses: This study examined reproductive function in three generations of rats and included a histopathological evaluation of reproductive organs in a limited number of F_2 and F_3 animals (5 sex/dose). Limitations of the study included no information about the numbers of F_1 and F_2 animals mated, no information about histological procedures, inadequate reporting of histopathological findings, and no sperm measurements.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for demonstrating no effects on reproductive function at doses up to 1000 mg/kg bw/day.

In an evaluation of a reproductive and developmental toxicity screen Harris et al. *(100*) tested ethylene glycol in 14-16-week old Swiss Crl:CD-1 mice. Ten male and female mice/group were gavaged with ethylene glycol in water at 0, 250, 700, and 2500 mg/kg bw/day. Aliquots of ethylene glycol were analyzed by gas chromatography after dosing and found to be 93-100% of the target dose concentration. The rational for dose selection was to achieve a high dose that was about 1/3 the level of the reported LD50. There were two separate groups of females in these studies. Group A females were dosed daily on study days 0 through study day 21. On study days 8-12 they were cohabitated with treated males. Percentage pregnant and number of live implants were the only endpoints examined in Group A. In Group B, time mated pregnant mice were dosed daily with ethylene glycol on GD 8-14 and followed until postnatal day 4. Fertility rate, bodyweight, litter size, and implantation sites were recorded. Males were treated daily from study day 3-20 and upon necropsy, liver kidney and testis weight was recorded as was epididymal sperm counts and motility. Data were analyzed by the Cochran-Armitage test for linear trend, Fisher's exact test, Kruskal-Wallis ANOVA, and/or Jonckheere's test for dose-response trends. Treatment of Group A females with 2500 mg/kg bw/day resulted in significant reductions in live implants/female and increases in the number of dead implants/female. Reduced litter weight on pnd 1 and 4 was the only significant effect noted in Group B females treated with 2500 mg/kg bw/day. Evaluation of males on study day 20 revealed no effect on sperm count or motility. Testis and epididymis weights were unaffected. There were no treatment related lesions in the testis and epididymis (preserved in Bouin's solution) or liver and kidney of males. Authors noted that the screening assay was less sensitive in detecting reproductive toxicity than continuous breeding studies such as the one conducted by Gulati et al. *(94*).

Strengths/Weaknesses: This study was clear in its design and the data are well reported. Effects, presented as reduced number of live implants and reduced total litter weight, were only seen at the 2500 mg/kg bw dose. The study also identified lack of histopathologic effect in kidney and testis at doses up to 2500 mg/kg bw.

Utility (Adequacy) for CERHR Evaluation Process: Results support findings of other studies.

The estrogenicity of ethylene glycol was examined by Ren et al. *(101)*. In an experiment that was replicated three times, immature rainbow trout (one/dose, 11.3-16.9 g) were ip injected with 10, 50, 100, 150, or 200 μL ethylene glycol (0, 0.99, 4.18, 9.43, 13.36, and 18.40 mg/g bodyweight). Control groups were injected with water only. Fish were sacrificed 24 hours post-injection, and livers examined for evidence of vitellogenin gene expression. Vitellogenin and estrogen receptor gene expression increased following ethylene glycol treatment. Ethylene glycol produced a dose-related effect upon estrogen receptor gene formation. At a dose of 9.43 mg/g bodyweight, it was found that ethylene glycol produced a weak estrogenic effect.

Strengths/Weaknesses: The sex of the fish was not specified but it was stated that neither male nor female immature trout express vitellogenin. There was no mention of a positive control such as estradiol.

Utility (Adequacy) for CERHR Evaluation Process: This study does not provide much insight on ethylene glycol other than to suggest it may have weak estrogenic effects in fish. The study is of limited utility due to incomplete reporting.

4.3 Utility of Data

4.4 Summary

Human Data

No human data were identified.

Experimental Animal Data

Results of the key animal reproductive toxicity studies are outlined in Table 4-4.

Mice. In two continuous breeding studies, CD-1 mice were exposed to 410-2826 mg/kg bw/day ethylene glycol in drinking water for 1 week prior to mating, 14 weeks of cohabitation and the entire gestation and lactation period *(94, 95)*. No adverse effect on fertility was noted in 2 generations of mice in either study. A slight reduction in the number of F_1 pups/litter following exposure to 1640 mg/kg bw/day in one study *(95)* was not repeated in the second study *(94)*. One study *(94)* included an evaluation of estrous cycles, reproductive organ histopathology in control and high dose animals, and sperm parameters in control and high dose F_0 males and F_1 males from all dose groups. No effects were noted for estrous cycles and histopathology of ovary, uterus, or vagina. A non-dose-related reduction in sperm count was observed in F_1 animals exposed to ≥897 mg/kg bw/day, with statistical significance obtained only in the two lower dose groups. Percent motile sperm were significantly reduced in F_1 mice exposed to ≥1798 mg/kg bw/day and abnormal sperm were increased in F_0 mice of the 2826 mg/kg bw/day group. Interstitial cell hyperplasia and epididymal lesions were increased in both generations of mice exposed to 2826 mg/kg bw/day compared to control mice. The Expert Panel determined that the

toxicological significance of seminiferous tubule degeneration was inconclusive due to the high incidence observed in control mice.

Histopathological results in a reproductive toxicity screen *(100*) support the conclusion that ethylene glycol produces no major reproductive toxicity effects in males. The study found no effects on sperm count and sperm motility and no treatment related lesions in the testis and epididymis of males gavage dosed with up to 2500 mg/kg bw/day ethylene glycol for 18 days.

Reproductive organ histopathology examined in subchronic and chronic toxicity studies (described in Section 2.2 and 2.4) found no treatment related histopathology of reproductive organs including ovary, uterus, prostate, testis, seminal vesicles and/or epididymis *(23, 62*). The results in female mice are consistent to those observed in the continuous breeding study *(94*). The lack of testicular lesions in subchronic and chronic studies *(23, 62*) suggests that the inconclusive finding of increased testicular lesions in the Gulati et al. *(94*) study was not treatment related. Interstitial cell hyperplasia and epididymal lesions observed in the breeding study were not observed in the chronic study despite higher concentrations and duration of dosing.

The Expert Panel concluded that data in mice are sufficient to demonstrate no effect on fertility of male or female mice following oral exposure to up to 2826 mg/kg bw/day ethylene glycol for approximately 22 weeks.

Rats. A multigeneration study examined reproductive toxicity in three generations of male and female Fischer 344 rats exposed to 40-1000 mg/kg bw/day ethylene glycol *(98*) and found no effect on fertility, gestation, offspring survival, or postnatal pup weight gain. No histopathological lesions were observed in reproductive organs (epididymis, testes, uterus, ovaries) of F_2 parents and F_3 offspring, although it was noted that histopathological procedures and findings were not adequately reported in the study. Estrous cycles and sperm parameters were not evaluated.

Results of histopathology evaluations (Section 2.2 and 2.4) conducted primarily in control and high dose animals in chronic and subchronic studies are consistent to findings observed in the multigeneration generation study.

The data are sufficient to demonstrate that ethylene glycol is not a reproductive toxicant in male and female rats following dietary exposure to up to 1,000 mg/kg bw/day for 7 weeks prior to mating in parental rats or from the time of conception through mating in offspring.

Table 4-4. Summary of Key Reproductive Toxicity Studies

Dose (mg/kg bw/day	Exposure Regimen	Species/ Strain	Dose (mg/kg bw/day): Effect	Reference
410 840 1640	In drinking water from 1 week prior to mating, 14-weeks of cohabitation, and throughout gestation and lactation	CD-1 Mouse	**NOAEL=840 mg/kg bw/day** **1640:** ↓No. F_1 litters/F_0 pairs ↓No. live F_1 pups/litter ↓F_1 pup weight No reproductive effects in F_1 adults	Lamb et al. *(95)*
897 1798 2826	In drinking water from 1 week prior to mating, 14-weeks of cohabitation, and throughout gestation and lactation	CD-1 Mouse	**897:** ↑No. F_1 litters/F_0 pairs, ↓F_1 sperm count, ↓F_1 testis and seminal vesicle weight ↓F_1 and F_2 pup weight **1789:** ↓F_1 sperm count and motility, ↓F_1 testis, seminal vesicle weight, and epididymis weight, ↓ F_1 relative (to bodyweight) testis and epididymis weight, ↓F_1 and F_2 pup weight **2826:** ↑Abnormal sperm in F_0, ↓F_0 and F_1 sperm motility, ↓F_1 testis, seminal vesicle weight, and epididymis weight, ↓F_1 relative (to bodyweight) testis and epididymis weight, ↑F_0 and F_1 testicular and epididymal lesions, ↓ F_0 bodyweight (M), ↑ F_0 kidney lesions and oxalate crystals (M), ↑F_1 death(M), ↓F_1 and F_2 pup weight, ↓ F_1pups/litter No effects were noted for fertility index, estrous cycles, or histopathology of female reproductive organs in either generation.	Gulati et al. *(94)*
250 700 2500	Females were gavage dosed on study days 0-21 and males on study days 3-20. Mating occurred on study days 8-21.	CD-1 Mouse	**2500:** ↓ Live implants and ↑ dead implants/female No effect on sperm count and sperm motility, and no treatment-related lesions in the testis, epididymis, liver or kidney of males.	Harris et al. *(100)*
40 200 1000	In diet continuously for three generations	Fischer 344 Rat	**NOAEL=1000** No effects on fertility, gestation index, gestation survival, histopathology of reproductive organs, or pup weight gain.	Depass et al. *(98)*

5.0 SUMMARIES, CONCLUSIONS, and CRITICAL DATA NEEDS

To be completed during the Expert Panel meeting.

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

5.2 Summary of Human Exposure

5.3 Overall Conclusions

5.4 Critical Data Needs

6.0 REFERENCES

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